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Science

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Science

COVER Sharks and other predators survey circling fish near Cocos Island in the eastern tropical Pacific Ocean. Overall, the species density of ocean predators is declining, but there are several remaining biodiversity hotspots that represent important targets for high-seas conservation efforts. See page 1365. (Photo: Bob Cranston, www.norbertwu.com)

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DEPARTMENTS

NEWS OF THE WEEK

NEWS FOCUS

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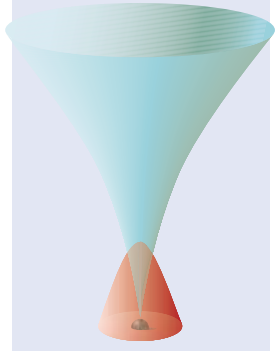

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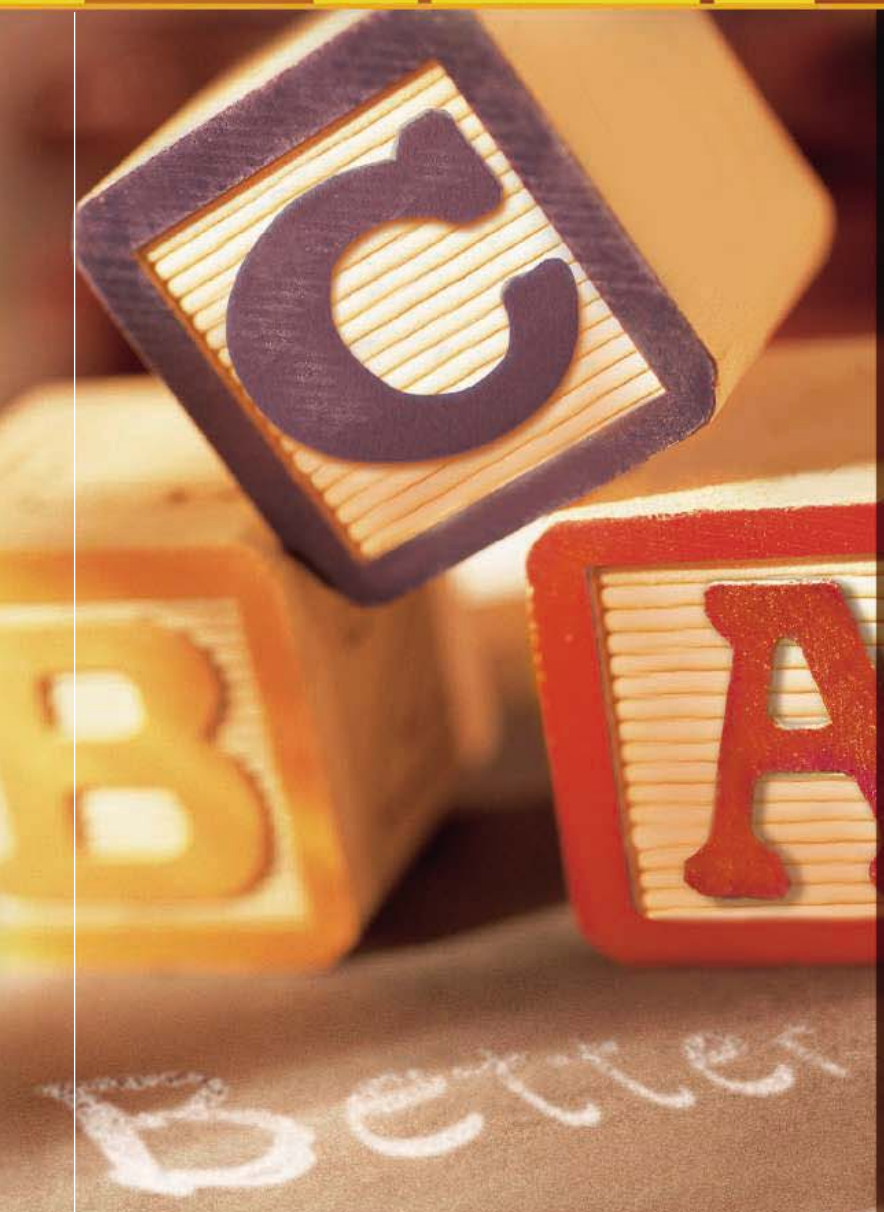

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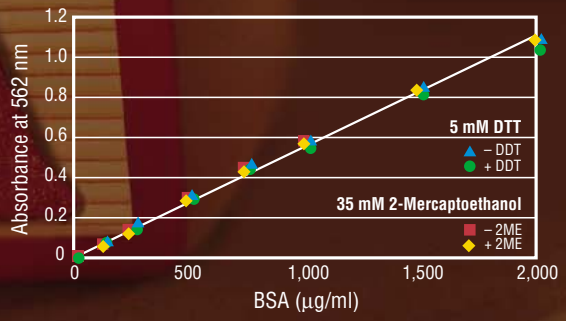

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PHYSIOLOGY: Suppression of Aging in Mice by the Hormone Klotho

H. Kurosu et al.

A fragment of a membrane protein circulating in the blood of mice increases life span when it binds to a cell surface receptor for insulin and insulin-like peptides. *related News story page 1310*

ECOLOGY: Phenotypic Diversity, Population Growth, and Information in Fluctuating Environments

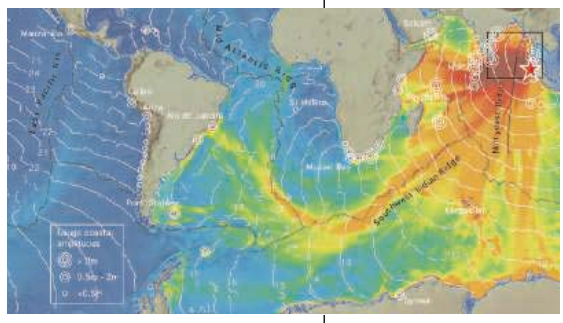
E. Kussel and S. Leibler

If their environments change rarely, the best strategy for bacteria is to switch phenotypes infrequently; if change is common, it is better to adapt accordingly.

OCEAN SCIENCE: The Global Reach of the 26 December 2004 Sumatra Tsunami

V. Titov, A. B. Rabinovich, H. O. Mofjeld, R. E. Thomson, F. I. González

A global model of the 2004 Sumatra tsunami shows that the waves were guided by Earth's mid-ocean ridges, explaining large waves in Peru and northeastern Canada 1 day later.



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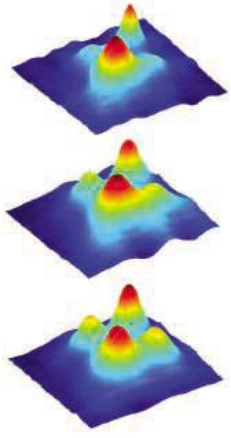

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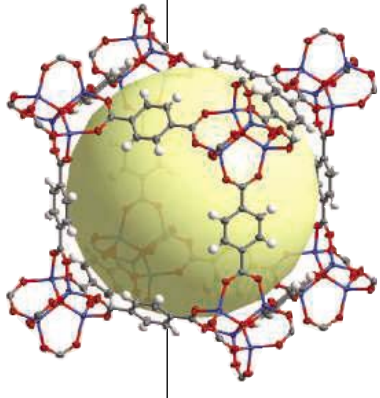

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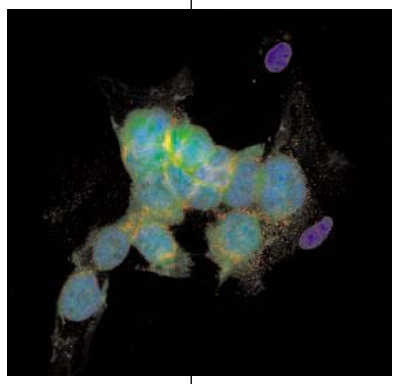

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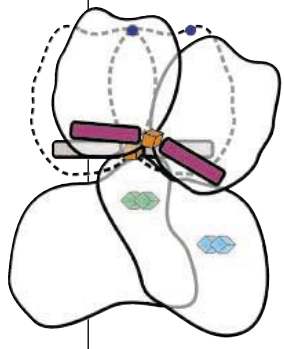

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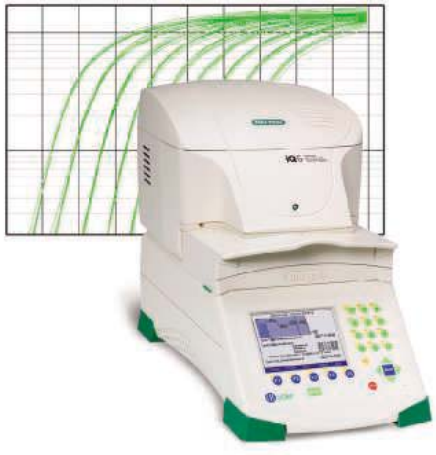

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science's next wave www.nextwave.org Career Resources for Young Scientists

US: An Insider's Guide to Washington, D.C. *Edited by J. Austin*
Science magazine and Next Wave staffers recommend their favorite fun places in Washington, D.C.

US: Educated Woman, Chapter 42—Fear and Loathing in Las Laboratory, Part 2 *M. P. DeWhyse*
Micella recounts advice from readers and reemphasizes the need for mentoring in graduate school.

MiSciNet: Congress Reduces the 2006 NSF Science Workforce Budget *C. Parks*
NSF's Education and Human Resources department will experience a 4.1% budget cut in the 2006 fiscal year and minority students may suffer as a result.

Weblog: European Science Careers News Clips *E. Pain and A. Forde*
Read about new bursaries for women in science and other funding, training, and job market news.

Weblog: USA Careers in Science Web Log *J. Austin*
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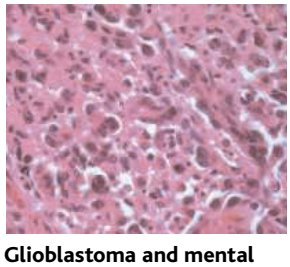
Case Study: Brain Tumor–Associated Dementia *J. McC. Noble, P. Canoll, L. S. Honig*
This study describes an unusual cause of cognitive decline.

News Focus: Tuning Up the Pancreas *M. Leslie*
Mammalian version of yeast longevity protein boosts efficiency of insulin-making cells.

News Focus: Family Feud *R. J. Davenport*
Protein relative of p53 has opposite influence on aging.



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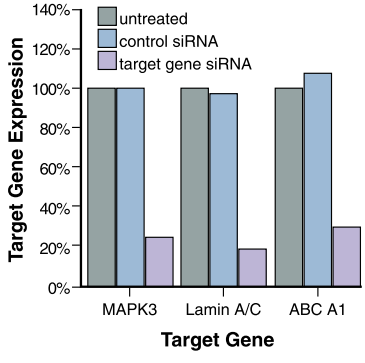

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Echoes of Phenol

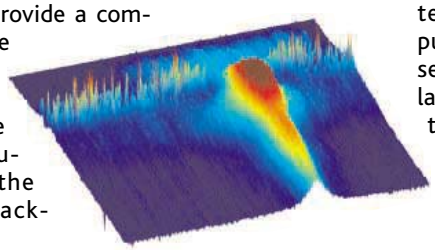
Multipulse nuclear magnetic resonance (NMR) techniques have long been used to study chemical equilibria in solution on time scales approaching microseconds. The advantage of NMR is that the induced nuclear spin dynamics used to obtain rates do not perturb the underlying chemistry of the system. **Zheng *et al.*** (p. 1338, published online 4 August 2005; see the Perspective by Dlott) show that an infrared analog of multipulse NMR, termed vibrational echo correlation spectroscopy, can raise the time resolution for such studies by more than six orders of magnitude. They quantify the picosecond time scale for association and dissociation of phenol-benzene complexes by relying on molecular vibrations, rather than nuclear spins, to track ensembles of exchanging molecules in solution.

Turning Slightly Faster

Several studies during the past 10 years suggest that Earth's inner core is rotating faster than the rest of the planet, but other studies have challenged these interpretations. Confirming super-rotation is important for understanding Earth's angular momentum and the generation of the magnetic field in the fluid outer core. **Zhang *et al.*** (p. 1357; see the news story by **Kerr**) have now analyzed 18 seismic doublets—nearly identical earthquakes that occur in the same place but separated by several to up to 35 years. A systematic offset in seismic waves that pass through the inner core demonstrate that it is indeed rotating faster than the rest of the planet by about 0.009 second per year.

Singlet-Triplet States in the Mix

The coupling of spins between adjacent quantum dots can form the basis of a quantum logic gate. However, recent work has shown that dots grown on GaAs also experience a large and random background field caused by the nuclear spins in the substrate, which leads to the spins losing their memory and mixing between spin-singlet and spin-triplet states. **Koppens *et al.*** (p. 1346) provide a comprehensive study of the extent of this effect and show how decoherence can be mitigated to some degree by tuning the coupling strength between the dots or polarizing the background nuclear spins.



CREDITS (TOP TO BOTTOM): ANDREW BAIRD; KOPPENS *ET AL.*

Ecological Community Structure Emerges

Efforts to understand ecological community structure and function have been hampered by debates about the shape of species numerical abundance and species resource-use curves. Much confusion has arisen from a combination of two factors: limited data and limited power to detect differences between model fits. **Connolly *et al.*** (p. 1363) overcome both limitations by applying information theory model-selection procedures to a large data set of tropical corals and reef fishes. Both resource-use and numerical abundance distributions are well characterized by a log-normal distribution. The distribution shape emerges at markedly different scales for resource-use and numerical abundance distributions. The scales at which log-normal distributions of numerical abundance become apparent are similar for two groups of organisms that differ markedly in dispersal and demography (corals and fishes). The large scale at which relative abundance patterns emerge indicates that the scale and scope of coral reef conservation strategies are inadequate, highlighting the need integration and networking of Marine Protected Areas regionally, across national boundaries.

Superconductivity Makes a Reentrance

How ferromagnetism and superconductivity can coexist in some metals has not been clear. **Lévy *et al.*** (p. 1343; see the Perspective by **Mackenzie and Grigera**) report that the superconducting ferromagnet, URhGe, enters a second superconducting phase at high magentic fields that are well above the region where superconductivity is destroyed. Magnetic torque and transport measurements suggest that superconductivty in this material is mediated by rotation of the magnetization.

Close Encounters on the Catalytic Kind

The enzyme nitrogenase catalyzes the reduction of atmospheric N_2 to ammonia (NH_3), which requires the input of six electrons (in addition to three protons). The electrons are carried on the iron-sulfur cluster of the Fe-protein and transferred to the MoFe-protein (where N_2 reduction takes place) in a reaction that depends on the hydrolysis of adenosine triphosphate (ATP). **Tezcan *et al.*** (p. 1377) provide three crystal structures of the complex of the Fe-protein and MoFe-protein in three distinct nucleotide states: (i) with no nucleotide bound, (ii) with adenosine diphosphate bound, and (iii) with an ATP analog bound. Taken together, these snapshots show that electron transfer is greatly facilitated as the Fe-protein crawls through the three nucleotide states, where the ATP state is the only one that allows for a sufficiently close approach of the iron-sulfur cluster and the recipient P cluster of the MoFe-protein.

Predator Diversity in the Oceans

The diversity of large ocean predators will vary in relation to temperature and ocean productivity. **Worm *et al.*** (p. 1365; published online 28 July 2005; see the cover) used extensive data sets from fisheries records to determine how the diversity of large predatory species (tuna and billfishes) varies throughout the world's oceans. Overall, there has been a decline in diversity during the past 50 years. The detailed analysis reveals peaks in diversity that occur at intermediate latitudes. Temperature and dissolved O_2 were the primary environmental factors

CONTINUED ON PAGE 1299

Illuminate the Mystery of Biological Dark Matter

First referred to as the "biological equivalent of dark matter" in the October 26, 2001 issue of *Science**, microRNAs (miRNAs) are small, highly conserved RNA molecules that act as key regulators of development, cell proliferation, differentiation, and cell cycle. miRNAs have been implicated in oncogenesis and viral infection. Explore this emerging field with a complete portfolio of innovative products specifically designed for miRNA investigation.

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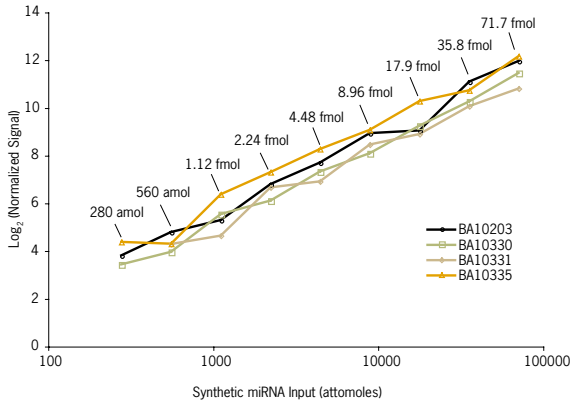

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* Ruvkun, G. 2001. Glimpses of a tiny RNA world. *Science* 294(Oct. 26):797-799.

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that correlated with (and may cause) the peaks in diversity. The abundance of zooplankton is highest in these "hot spot" regions, which suggests that diversity peaks may be similar for organisms throughout the marine food chain.

Stem Cell Research sans Embryos?

The use of human embryos has triggered considerable societal debate about human embryonic stem (hES) cell research. **Cowan *et al.*** (p. 1369) describe an alternative method of deriving hES cells that may ultimately eliminate the need for human embryos and oocytes. Experimentally induced fusion of human adult somatic cells with hES cells in culture produces hybrid cells that are transcriptionally "reprogrammed" back to the embryonic state. If future experiments indicate that this reprogrammed state is retained after removal of the pluripotential ES cell nucleus (currently a formidable technical hurdle), the hybrid cells theoretically could be used for the production of genetically tailored hES cell lines.

CREDIT: KÖRNER *ET AL.*

Results from the Canopy

To understand the effects of rising levels of atmospheric CO_2 levels on trees, there have been a number of free-air CO_2 enrichment (FACE) experiments in recent years, mostly in young plantations. **Körner *et al.*** (p. 1360; see the news story by **Pennisi**) assessed the responses of mature trees in a near-natural temperate forest using a system that delivered CO_2 to the crowns of 35-meter-tall trees. After 4 years, different tree species had different responses to higher CO_2, but one common response was a lack of sustained growth stimulation. Thus, carbon appears to pass through the system at a greater rate when CO_2 levels are higher.

Regulated Regulation in Immune Responses

The activity of regulatory T cells (Treg) is responsible for controlling aberrant immune responses and autoimmunity, but these cells represent a potential barrier to certain types of therapeutic manipulation, such as in cancer immunotherapy. **Peng *et al.*** (p. 1380) provide evidence that part of human Treg control may be mediated directly by an innate signaling protein. Clones of human Treg cells, as well as isolated, naturally occurring Treg cells, expressed Toll-like receptor (TLR) 8. Ligands that could activate this receptor reversed the suppressive activity of these cells in culture, as well as in a mouse tumor model. Control over Treg activity via TLR signals may open new avenues for inhibiting unwanted immune suppression during cancer immunotherapy.

Secrets of Malaria Invasion

The parasite that causes cerebral malaria, *Plasmodium falciparum*, can switch the host receptors used for invasion of human red blood cells. This property has been known for more than 10 years but the underlying mechanism has been unclear. Using microarrays and gene knockouts, **Stubbs *et al.*** (p. 1384) have identified the *PfRh4* gene as responsible for switching. This mechanism would be important for the parasite population to avoid host immune responses and erythrocyte polymorphisms, and has important implications for vaccine design.

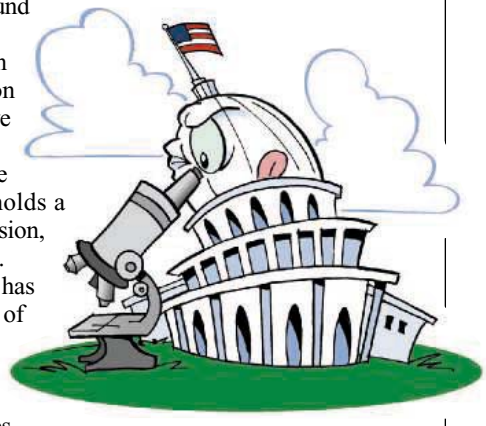
Millions of Microbe Species, If Left Alone

The traditional methods of calculating diversity by identifying and counting organisms fail for microbes. We do not know how many species of microbes there are, even to within a few orders of magnitude. **Gans *et al.*** (p. 1387; see the Perspective by **Curtis and Sloan**) used a method based on historical data for DNA reassociation kinetics. The results are startling: The method suggests that there are about one million species in a pristine environment; most of which are quite rare. This number represents an increase of two orders of magnitude compared to first estimates. The work also highlights the dramatic effects of pollution on diversity: the presence of toxic metals extirpates the rare species leading to loss of 99% of the original diversity.

Silly Season on the Hill

What in the world is going on with the U.S.Congress? Back in the "old days" of the 1970s, members of the House and Senate didn't have much personal interest in science. Occasionally, an expert in some field was elected and played a useful role in science policy, like astronaut-geologist and former Senator Harrison Schmitt, who kept an eye on space exploration issues. But most have shown their interest by giving the National Institutes of Health more money than requested by the administration annually, even tacking on a few special science facilities for their state's medical school. It may have been pork, but at least it was kosher.

But now, in the silly season of August, it seems that nearly everybody on Capitol Hill is knee-deep in science! Members suddenly know how to evaluate individual grants, even defunding those that deal with touchy subjects. One banned grant dealt with the psychology of romance—apparently too hot to handle these days. A number of current legislators have also become amateur neurobiologists, developing an unexpected command of difficult topics like "persistent vegetative state." The Senate's chief surgeon, Dr. Bill Frist (R-TN) established a record for definitive long-range TV diagnosis on that subject. Then, thankfully, he staged a dramatic turnaround on stem cell research. We never know exactly what to expect from these guys.



It's reassuring that genuinely well-qualified scientists persist in a few refuges on the Hill. One physicist in Congress, Rush Holt (D-NJ), is an example. His work on education and the support of science funding has been exemplary, and it's good to have an expert with his credentials on the Intelligence Committee. And there are able Republicans in the serious science game as well. The chairman of the House Science Committee, Sherwood Boehlert (R-NY), is one; his colleague Vern Ehlers (R-MI) holds a doctorate in physics. That committee has stuck thoughtfully to its jurisdiction and mission, and the science community should be grateful for its upgraded substantive leadership.

But one congressional committee has become so enthusiastic about science that it has strayed off the reservation into unclaimed territory. Chairman Joseph Barton (R-TX) of the House Committee on Energy and Commerce has sent demand letters to a number of people: Dr. Rajendra Pachauri, chairman of the Intergovernmental Panel on Climate Change (IPCC); Dr. Arden Bement Jr., director of the National Science Foundation (NSF); and research professors Drs. Michael E. Mann, Malcolm K. Hughes, and Raymond S. Bradley, who collaborated on recent analyses of global temperature proxy data. The text of each letter begins with a brief summary of the conclusions of the IPCC regarding human influence on recent global warming. Then, after reciting some reasons for skepticism about those conclusions and Dr. Mann's role in them, it lists an extraordinarily burdensome set of demands.

These include disclosure of all funding sources, agreements regarding that support, exact computer codes, locations of data archives used, responses to referenced criticisms of the work, and the results of all temperature reconstructions. That's only the beginning. The letter to Dr. Mann contains highly specific requests spanning 8 paragraphs and 19 subparagraphs. Dr. Bement's letter demands exhaustive lists of all agency policies, all grants related to climate research, policies relating to IPCC review, information regarding requests for access to research records, and more. It's clear that what's going on here is harassment: an attempt at intimidation, carried out under a jurisdiction so elastic that any future committee chair might try to play this game if coached by the right group of unschooled skeptics.

There are ways of avoiding both the harassment and the precedent. Chairman Boehlert could take charge of matters, because this debate belongs with the real science committee. If hearings are necessary, they can be held. If independent and objective information is needed, the Congressional Research Service could help. Better still is the time-tested way of reaching scientifically sound conclusions: scientific experiment, analysis, debate, and review. A letter* to Chairman Barton from *Science*'s publisher, the American Association for the Advancement of Science, points that out in prose more tactful and elegant than I can presently manage. As for me, I'm just the editor—and I'm outraged at this episode, in which science becomes politics by other means.

Donald Kennedy
Editor-in-Chief

*www.aaas.org/news/releases/2005/0714letter.pdf

10.1126/science.1117863

ILLUSTRATION: PAT N. LEWIS

edited by Gilbert Chin

OPTICS

Optical Conservation

A 50-year-old test painting and a spot (inset) where new varnish was applied over old.

In the interests of conservation, historical research, and attribution, paintings in museums may be subjected to a barrage of scientific probes, each of which is sensitive primarily to surface or subsurface features; sometimes, small samples are physically removed from the painting for analysis. The development of techniques that are nondestructive and noninvasive is not only desirable but also necessary when it comes to examining old and delicate pieces. The optical interferometric technique of optical coherence tomography (OCT) is usually associated with the three-dimensional imaging of biological samples, particularly the inner structure of the eye. Liang *et al.* show that OCT can also be used for the noninvasive examination of paintings to provide high-resolution and dynamic imaging capabilities for visualizing the structures of layers of varnish, layers of paint, and even the preliminary sketches underneath. This imaging technique should prove to be a useful tool for the conservation and attribution of art. — ISO

Opt. Express **13**, 6133 (2005).

CELL BIOLOGY

A Tale of Two Signals

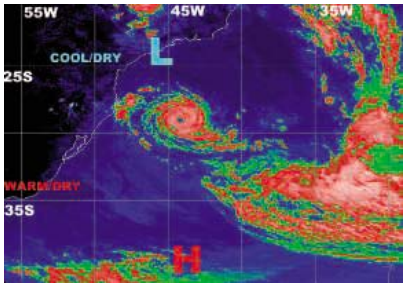
The intracellular transport of membrane proteins requires cellular machinery that recognizes targeting signals that may be present within the cytoplasmic, membrane, or extracellular domains of the protein. But some proteins contain multiple targeting signals, which need to be decoded sequentially to execute the correct protein itinerary. Anderson *et al.* have examined the signals in NgCAM, a cell adhesion molecule that is generally found in the axonal membrane of neurons, but is first transported to the dendrites. When expressed in an epithelial cell line, NgCAM is transported to the basolateral plasma membrane and then transcytosed to the apical surface, where it remains despite multiple rounds of endocytosis and reinsertion into the apical membrane. Why then, after endocytosis, does the protein not go back to the basolateral surface? The signal for basolateral targeting resides in the cytoplasmic domain of NgCAM and is recognized by an adaptor protein that ensures delivery of newly synthesized protein to the basolateral surface. This signal is masked by phosphorylation of a key tyrosine residue, which uncovers a cryptic apical targeting signal in the extracellular domain and also maintains the protein within a recycling cycle at the apical surface. — SMH

J. Cell Biol. **170**, 595 (2005).

CLIMATE SCIENCE

The First of Many?

The first hurricane ever documented in the South Atlantic, Catarina, struck the southern coast of Brazil on 28 March 2004. This unprecedented event led some Brazilian meteorologists to deny that it was a hurricane at all; further analysis, however, has shown that it was.



Satellite image showing Catarina approaching Brazil, 26 March 2004.

In a detailed study of the storm, Pezza and Simmonds describe its evolution from genesis on 20 March 2004 as an extra-tropical cyclone, through its strengthening to a category I hurricane before it drifted over land. This hurricane developed because of an unusual combination of high sea surface temperatures, low vertical wind shear, and strong mid-to-high latitude blocking (which interferes with normal east-west atmospheric flow). These conditions are functions of large-scale atmospheric circulation patterns in the region and could be related to climate change. If so, more hurricanes may occur in the South Atlantic in the future. — HJS

Geophys. Res. Lett. 10.1029/2005GL023390 (2005).

IMMUNOLOGY

Returning the Complement

Many an immunology undergraduate's headache can be traced to memorizing the intricacies of the complement system. Three activation pathways lead to the generation of the C3 converting enzymes, which are responsible for generating the effector molecules that carry out crucial host defence functions. As a result, the complement system is a target for viral and bacterial evasion strategies.

The bacterial pathogen *Staphylococcus aureus* has evolved a bacteriophage-encoded pathogenicity gene cluster (SaP15) that is present in 90% of strains and encodes four secreted human-specific virulence proteins. Rooijakkers *et al.* observed that one of these, designated SCIN, inhibited bacterial phagocytosis by human neutrophils, by blocking the deposition of the complement factor C3b on bacterial membranes, which is a crucial step in opsonization. Further upstream, SCIN could inhibit all three pathways by binding to the C3 convertases (C4b2a and C3bBb). Potentially, such interactions could alter the intrinsic decay potential of the convertases, which activate downstream effector molecules of the complement pathway. As a consequence, SCIN has the ability to interfere with the complement system at multiple points, making it a drug development target for diseases involving aberrant complement activity. — SJS

Nat. Immunol. 10.1038/ni1235 (2005).

MATERIALS SCIENCE

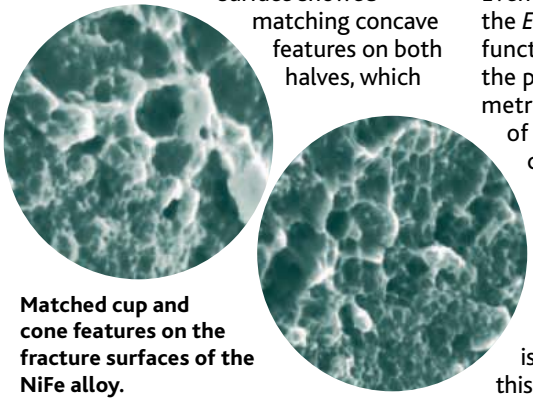
A Sizeable Break

Metals and alloys containing nanocrystalline-sized grains are of interest because of their superior strength, wear

CREDITS: (TOP) LIANG *ET AL., OPT. EXPRESS* **13**, 6133 (2005); (BOTTOM) PEZZA *ET AL., GEOPHYS. RES. LETT.* 10.1029/2005GL023390 (2005)

resistance, and superplasticity, which is the ability to deform a material beyond its usual breaking point. When nanostructured metals are defect-free, they also show reasonable tensile elongations in addition to their enhanced strength. However, as the grain size decreases, the mechanism of plastic deformation changes from one that is dislocation-mediated to one that is grain boundary–mediated; it is not known if the failure mechanism changes from ductile to brittle, which might limit the applicability of these materials.

Li and Ebrahimi examined nanocrystalline nickel and nickel-iron alloys with grain sizes above and below the critical size, respectively. In tensile testing, the Ni specimen showed significant necking before fracture, indicative of ductile behavior. Examination of the fracture surface showed matching concave features on both halves, which



Matched cup and cone features on the fracture surfaces of the NiFe alloy.

is consistent with the formation of microvoids during deformation. In contrast, the NiFe alloy showed little necking, indicative of a much lower toughness. The fracture surface showed a cup and cone pattern, or a series of voids and protrusions. The authors attribute this cup and cone pattern to the meandering of the path, and hence the fracture to the breakage of atomic bonds rather than cavity growth. — MSL

Adv. Mater. **17**, 1969 (2005).

BIOCHEMISTRY

Two by Two

Membrane proteins have never been easy to study, but two groups have applied modifications of soluble protein biochemistry to catalog protein-protein interactions in the membranes of *Escherichia coli* and *Saccharomyces cerevisiae*. Stenberg *et al.* use a two-dimensional (native/denaturing) electrophoretic system to identify 34 solubilized protein complexes from the bacterial inner membrane and 9 complexes from the outer membrane. Even though the complete sequence of the *E. coli* genome is available, the functional roles of many genes are not; the protein YhcB associates stoichiometrically with the two major subunits of cytochrome bd quinol oxidase and can now be assigned as a subunit of this enzyme. Miller *et al.* use the split-ubiquitin yeast two-hybrid system to enumerate almost 2000 interactions involving roughly 500 integral membrane proteins. Unlike the stable complexes isolated by detergent solubilization, this approach probably picks up transient interactions as well, and correlating the two-hybrid results with bioinformatic and experimental data led to their classification into confidence categories, of which 131 interactions were most likely to represent true positives. — GJC

J. Biol. Chem. 10.1074/jbc.M506479200 (2005); *Proc. Natl. Acad. Sci. U.S.A.* **102**, 12123 (2005).

HIGHLIGHTED IN *SCIENCE*'S SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

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Spliced in the Cytoplasm

In nucleated cells, noncoding introns are removed from pre–messenger RNA (pre-mRNA) transcripts by the spliceosome (a nuclear complex of proteins and RNAs) before export of the mRNA from the nucleus. Thus, one would not expect that platelets—anucleate blood cells that bud from megakaryocytes—would contain spliceosome components or pre-mRNAs. Nevertheless, Denis *et al.* show that components of the spliceosome are present in the cytoplasm of human megakaryocytes and also in circulating platelets. Interleukin-1β (IL-1β) pre-mRNA is present in the cytoplasm of quiescent platelets, whereas platelets that had been activated by adhesion to fibrinogen in the presence of thrombin contain mature IL-1β mRNA and protein. Moreover, IL-1β pre-mRNA could be converted into mature mRNA by a platelet extract. Thus, pre-mRNA splicing is a key regulatory point for cytokine production during platelet activation. — EMA

Cell **122**, 379 (2005).

CREDITS: LI *ET AL.*, *ADV. MATER.* **17**, 1969 (2005)

GENETIC IMAGINE SOLVING

YOUNG SCIENTIST AWARD

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DISEASE:
A 20-YEAR RIDDLE

Well that's just what one young scientist did when she unlocked the secrets of the spliceosome, a crucial molecular machine within the cell. Dr. Saba Valadkhan's breakthrough discovery won her the 2004 Young Scientist Award.

The spliceosome plays a key role in human health. Errors in its function are thought to cause up to 50% of all genetic disease – the tiniest mistake can result in retinal degeneration or neurological disease. A clear understanding of how this large and complex structure works had evaded scientists despite two decades of research. But Dr. Valadkhan has changed that with the successful development of a novel, minimal spliceosome stripped down to the core elements. This is now shedding light on how spliceosome errors translate into mistakes in gene expression.

Dr. Valadkhan won the grand prize in the 2004 Young Scientist Award competition with an essay based on her research in this area. She is now an assistant professor at the Center for RNA Molecular Biology at Case Western Reserve University in Cleveland, Ohio (USA). She says: "The prize has been very beneficial to my career. It has given me valuable new connections, and a great deal of recognition in the scientific community. It has also helped me see my work in a wider context, and understand what science is really all about."

YOUR OPPORTUNITY TO WIN IS NOW

The Young Scientist Award was established in 1995, and is presented by *Science*/AAAS and GE Healthcare. The aim of the prize is to recognize outstanding most recent Ph.D.s from around the world and reward their research in the field of molecular biology.

This is your chance to gain international acclaim and recognition for yourself and your faculty. If you were awarded your Ph.D. in molecular biology* during 2004, describe your work in a 1,000-word essay. Then submit it for the 2005 Young Scientist Award. Your essay will be reviewed by a panel of distinguished scientists who will select one grand prize winner and up to seven regional winners. The grand prize winner will get his or her essay published in *Science*, receive US$25,000, and be flown to the awards ceremony in St. Louis, Missouri (USA). Entries should be received by **September 30, 2005**.

Go to **www.aaas.org/youngscientistaward** to find the entry form. We wish continued success to Dr. Valadkhan. And to you.

Read Dr. Saba Valadkhan's latest findings in *RNA*. 2003 Jul, **9** (7): 892-904.

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imagination at work

* For the purpose of this prize, molecular biology is defined as "that part of biology which attempts to interpret biological events in terms of the physico-chemical properties of molecules in a cell" (*McGraw-Hill Dictionary of Scientific and Technical Terms*, 4th Edition).

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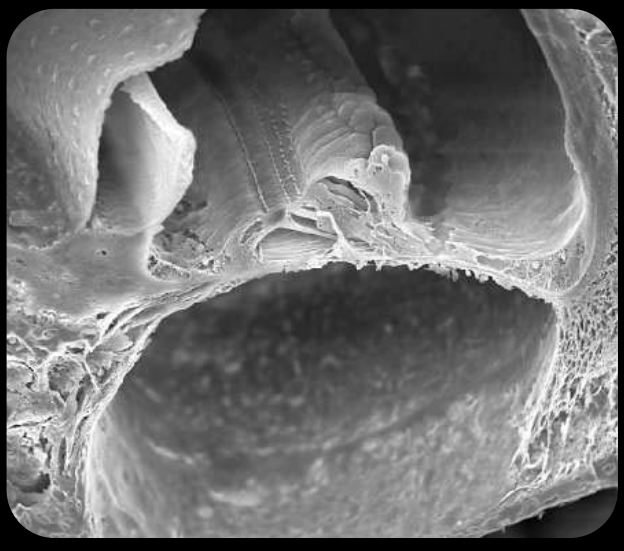


EDUCATION

Hearing Aid

Distinguishing the trill of a canary from the blare of a foghorn is a job for the cochlea, which transforms sounds entering the inner ear into nerve impulses that the brain can interpret. Students and researchers can study the cochlea's architecture and intricate workings at this detailed primer from Italian researchers Renato Nobili of the University of Padua and Fabio Mammano of the Venetian Institute of Molecular Medicine. The anatomy section dissects the coiled structure down to the vibration-detecting inner hair cells. Plentiful illustrations and animations can help you grasp the complexities of translating waves in the cochlea's fluid into nerve signals. The site also offers some aural history, highlighting pioneers such as Italy's Alfonso Corti, who first described the cochlea's internal organization. Above, a cross section through the cochlea shows the organ of Corti (center), which houses the hair cells.

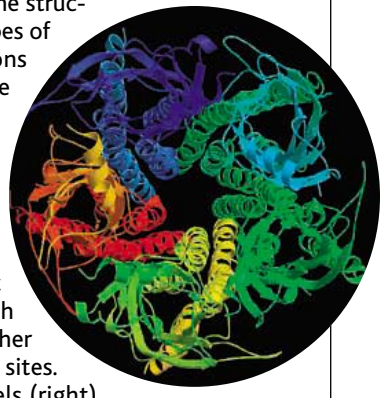
www.vimm.it/cochlea/index.htm

RESOURCES

Journey Through the Membrane

The cell membrane rebuffs ions such as sodium and potassium that attempt to traverse it. But the charged particles can enter and exit cells through protein tunnels known as ion channels that are embedded in the membrane. This trio of sites lets everyone from neophytes to neuroscientists boost their understanding of these passages, which are crucial for nerve cell firing and other activities.

Soak up the basics of ion channels at this tutorial* from Tim Smith, a chemistry student at the University of Warwick, U.K. The pages describe the structure and mechanics of different types of channels and explain how poisons such tetrodotoxin, produced by the puffer fish, can lock the cellular doorways. Ion Channels.org† is a community site for researchers and students. Sponsored by Ion Channel Media Group of Montreal, Canada, the site features an annoyingly large number of ads but also includes abstracts of fresh papers, a jobs board, and links to other ion-channel and electrophysiology sites. So-called ligand-gated ion channels (right) open or close when molecules such as the neurotransmitter acetylcholine latch on. This database,‡ hosted by the European Bioinformatics Institute, stows amino acid sequences for more than 500 components of ligand-gated channels from humans, mice, rats, and other organisms.



* www.chemsoc.org/exemplarchem/entries/2002/Tim_Smith
† www.ionchannels.org
‡ www.ebi.ac.uk/compneur-srv/LGICdb/LGICdb.php

DATABASE

When the Earth Moved

The 1964 Great Alaska earthquake toppled buildings in Anchorage, 120 kilometers from the epicenter, and touched off a 67-meter-high tsunami that killed 110 people. The database SeismoArchives houses recordings of the magnitude-9.2 temblor, the second largest of the 20th century, and 25 other "classic" quakes.

The goal of the clearinghouse, a new offering from the seismology consortium IRIS (*Science*, 26 November 1999, p. 1643), is to cache digital versions of deteriorating paper and microfilm seismograms. The hundreds of original recordings in the archive come from researchers and span nearly 70 years of ground shaking, from the 1906 Valparaiso, Chile, quake to the 1972 Managua, Nicaragua, disaster. Earth scientists who want to analyze the events can download high-resolution images of seismograms captured by stations around the world.

www.iris.edu/seismo

EXHIBITS

Science on Screen

Whether the character is Dr. Frankenstein, Dr. No, or Mr. Spock, scientists and mathematicians typically appear on TV and in film as megalomaniacs or maladjusted superbrains. Breaking those stereotypes is the goal of Science Cinémathèque, hosted by the Museum of the Moving Image in Astoria, New York. The exhibit, which premiered this week, explores more complex portrayals of research and researchers in popular culture. For example, you can screen eight prizewinning student films with scientific themes, including a short biopic on the Hungarian physician Ignaz Semmelweis (1818–1865), who demonstrated the importance of hygiene in hospitals. Other features include a panel discussion of the 2004 film *Primer*, about garage inventors who build a time machine (above).

www.movingimage.us/science

Send site suggestions to netwatch@aaas.org. Archive: www.sciencemag.org/netwatch

CREDITS (TOP TO BOTTOM): BECHARA KACHAR, NIDCD/NIH; PROTEIN DATA BANK; MUSEUM OF THE MOVING IMAGE

This Week

PAGE 1310
Buying time, at a price

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Hope deferred

INFECTIOUS DISEASES

WHO Probes Deadliness of China's Pig-Borne Disease

International experts fear that a new, more virulent form of the bacterium *Streptococcus suis* could be responsible for killing 38 humans and more than 600 pigs in China's central Sichuan Province over the past 2 months. But they are puzzled about how a rare—and rarely fatal—disease that usually appears in isolated cases among humans became so deadly and whether it might strike again.

Answering those questions will depend on strengthening collaborations between Chinese researchers and the international community. Additional animal epidemiological studies will be needed in China to determine if and how widely the new strain may be circulating. Jeff Gilbert, a zoonotic disease expert with the World Health Organization (WHO) in Manila, says, "from the human health side, (cooperation) has been fairly impressive, but we're still missing the veterinary information" on the outbreak.

A half-dozen experts on the disease joined technical staff from WHO and international animal health organizations in a private 9 August conference call to review information provided by China's Ministry of Health. The ministry reported that the outbreak peaked in mid-July and that no new cases were reported after 5 August. Of the 204 human cases, there were an unprecedently high 38 deaths. Nearly all patients were farmers or butchers who had slaughtered sick pigs or handled the meat.

Outbreak. Questions remain about the swine disease that has killed 38 people in China.

Tests on both human and animal samples confirmed the presence of *Streptococcus suis* serotype 2 and ruled out other bacterial and viral agents, including influenza and Nipah virus. The ministry found no evidence of human-to-human transmission. WHO reported publicly last week that experts now accept the ministry's conclusions.

"We have no doubt the identification is correct; it is *Streptococcus suis*," says Marcelo Gottschalk of the University of Montreal in Canada, who was initially skeptical because of the strange nature of the outbreak. The bacterium is endemic among domestic pigs worldwide but is usually asymptomatic. The Sichuan outbreak is by far the largest ever reported, surpassing a previous outbreak in China's eastern Jiangsu Province in 1998 that killed 14 of 25 human patients and caused the death or culling of 80,000 pigs. (Little is known of this outbreak outside of China because all scientific reports appeared in Chinese journals.)

Gottschalk says the mortality rate far exceeds the 5% to 6% typically seen among sporadic human cases. In addition, most recent victims succumbed to toxic shock, an atypical symptom of the disease. "It is logical to think that this is a more virulent strain that acquired genetic material from other microorganisms," Gottschalk concludes.

Xu Jianguo, director of the National Institute for Communicable Disease Control and Prevention, a lab affiliated with China's Center for Disease Control and Prevention (CDC) in Beijing, says that sequencing of Sichuan isolates has not turned up new genetic changes. He speculates that the outbreak arose because the type 2 serotype, known to be more virulent than other serotypes, may be becoming more widespread in pigs, increasing the chance of human infection.

To determine whether the bacterium has changed, researchers need to compare both ▶

HIGHER EDUCATION

Princeton Resets Family-Friendly Tenure Clock

Princeton University wants to level the field for tenure-track faculty members starting a family. Starting this fall, both men and women who become parents will receive an automatic tenure extension. This first-of-its kind policy is seen as one way to help boost the number of tenured women in science and engineering departments. But some say the policy could provide an unfair advantage to scholars who are not the primary caregivers.

Many universities, including Princeton, already allow new parents to request extra time for tenure decisions. But studies show that many women (and men) worry that asking might be seen as showing a lack of commitment to academic life (*Science*, 17 December 2004, p. 2031). "There is a feeling among assistant professors that stopping the clock could hurt your chances of getting tenure," says Princeton psychologist Joan Girgus, who chaired a 2003 campus report that recommended changing the current policy. Assistant professors at the university will now automatically receive one additional year for every child born or adopted, although they can request an early tenure review.

Lisa Wolf-Wendel, a sociologist at the University of Kansas in Lawrence who studies gender issues, says the impact of the new policy is hard to predict. "If going up early for tenure ends up becoming the norm, then you haven't solved the problem," she says, adding that the policy could end up favoring men with stay-at-home wives or partners who do the actual work of childrearing. "An extension would allow them to be more academically productive," she notes.

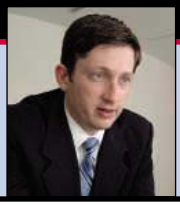
One solution, in the works at the University of California, would give automatic extensions to those with "substantial caregiving responsibilities," says Marc Goulden, an analyst at UC Berkeley's graduate division. The policy would require faculty members to submit a letter attesting to that status.

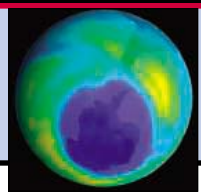
–YUDHIJIT BHATTACHARJEE

CREDIT: AP PHOTO

1314
Ecologists branch out

1319
A patent 'Robin Hood'





1320
Ancient Earth systems

human and animal isolates from the Sichuan outbreak with those collected previously within China and in other countries. Xu says discussions on international collaborations are underway. "I think China will be very open about sharing samples, but you need to go through the proper procedures."

WHO's Gilbert hopes that additional human epidemiological and clinical information is included in a paper China's CDC is reportedly now readying for an international journal. He applauds the Ministry of Health for keeping the international community informed of human cases but says the Ministry of Agriculture has not been as forthcoming. Specifically, he says it has failed to clarify such basic epidemiological features as how many pigs have died or been culled and the nature of the affected livestock operations. He adds that surveillance of pig farms may be needed to restore consumer confidence in the safety of pork products.

Meanwhile, officials in China's southern Guangdong Province recently reported four isolated human cases, including one death; all of the patients may have been exposed to infected meat. And experts are awaiting further details on the suspected infection of two butchers who died in early August in Jiangsu Province. Hong Kong also recently confirmed its tenth case this year, although it is not clear if there is a connection to the Sichuan outbreak. **–Dennis Normile**

SCIENTIFIC COOPERATION

Kashmir Workshop Aims to Break the Ice

Jack Shroder and Michael Bishop know that one scientific workshop next spring won't erase a half-century of rancor between India and Pakistan over Kashmir. But the two University of Nebraska geoscientists, just back from their latest expedition to the Himalayan region, believe that examining the scientific processes taking place at the rooftop of the world could not only ease tensions between these two bitter enemies but also advance science and benefit the people of South Asia. Thanks to $125,000 from two U.S. agencies and a private foundation, the two are preparing to take the first step toward turning the Karakoram mountain range and the nearby Siachen Glacier into a scientific peace park.

"It makes no sense to have troops there at 20,000 feet," says Shroder about the Siachen Glacier, the world's highest battlefield, where the harsh environment has claimed more lives than bullets have over 2 decades of sporadic warfare between the two countries. "If this could be turned into a peace park, then the military could leave and the scientists and mountain community could play." Adds Pervez Hoodbhoy, a physicist at Quaid-e-Azam University in Islamabad, Pakistan, "Bitter hatreds are giving way to a grudging acceptance of the other's existence. Suddenly everything has become possible."

The idea of turning the war-torn region into a peace park has been around for several years. But the concept began to gel 2 years ago after Harry Barnes, a former U.S. ambassador to India, contacted Shroder about organizing a workshop. Shroder used his 25-year scientific ties to the region to sign up Syed Hamidullah, director of the Centre of Excellence in Geology at the University of Peshawar in Pakistan, and Syed Iqbal Hasnain, vice chancellor of Calicut University in India.

This month, the National Science Foundation (NSF) awarded a $70,000 grant to what Schroder and Bishop have labeled the Karakoram Science Project. Combined with $30,000 from the Office of Naval Research and $25,000 from the Lounsbery Foundation, the money will enable some 30 to 40 scientists from the United States, India, Pakistan, China, and elsewhere to meet next May in Lahore, Pakistan, to discuss an array of geological, climactic, and environmental questions. "NSF was particularly interested in including younger scientists," says Shroder. "It's the first time they've ever given me more money than I've asked for."

High hopes. Jack Shroder (*left*) and Mike Bishop envision the Karakoram mountains as a magnet for scientists.

In June, Indian Prime Minister Manmohan Singh made an unprecedented visit to the site and proclaimed his support for making Siachen, the largest midlatitude glacier in the world, a mountain of peace. "The NSF grant is a step in the right direction," says Hasnain, "in building bridges that might lead to the ultimate demilitarization" of the glacier. Hoodbhoy believes that the workshop, if it leads to a peace park, is "proof that enmities are not forever."

Bishop and Shroder plan to concentrate on the science and leave the peacemaking to others. But they readily acknowledge that the workshop could be the start of something much bigger. "If we can get people to work together, there's no telling what could come of it," says Bishop. "We just want to get the ball rolling." **–Jeffrey Mervis**

With reporting by Pallava Bagla in New Delhi, India.

CREDITS (LEFT TO RIGHT): ANDY BUSH; JACK SHRODER

PHYSIOLOGY

Boosting Gene Extends Mouse Life Span

A protein named after the Greek goddess who spins life's thread has joined the short list of ways to extend a mouse's natural life span. Whereas lab mice can live about 2 years, mice engineered to overproduce this protein, called Klotho, have celebrated third birthdays, Makoto Kuro-o of the University of Texas Southwestern Medical Center in Dallas and his colleagues report online in this week's *Science* Express (www.sciencemag.org/cgi/content/abstract/1112766). The mutant rodents represent a rare case of a single gene substantially influencing life span in mammals.

"I'm not a dreamer; I don't think we're going to find a master control gene for aging," says Harry Dietz, a geneticist at Johns Hopkins University in Baltimore, Maryland, who studies Klotho's counterpart in humans. But, he says, "this is the next best thing. We have found something that perhaps has the ability to make old age richer."

But Kuro-o, who discovered the gene that encodes Klotho, worries that "too much Klotho might not be very good." The mice he created with extra Klotho look like animals at risk of diabetes. There's also disagreement over how Klotho works.

Mice lacking Klotho die young, after developing arteriosclerosis and other age-related conditions much earlier than normal (*Science*, 7 November 1997, p. 1013). Still, many doubted that extra Klotho would lengthen life span. With a short-lived mutant, "you always have to worry that it's just sick," says Cynthia Kenyon, who studies aging at the University of California, San Francisco.

Rare milestone. These mice, which overexpress the gene for Klotho, have celebrated their third birthdays.

So, Kuro-o, his postdoctoral fellows Hiroshi Kurosu and Masaya Yamamoto, and colleagues at universities in the U.S. and Japan created mice overexpressing the gene for Klotho. While Klotho is produced only in the kidney and brain, a fragment of it slips into the blood and may act like a hormone. Males making extra Klotho lived up to 30% longer than normal males, and the mutant females survived 20% longer than normal counterparts. As with lab animals coaxed to have lengthy life spans, the altered rodents had fertility problems. They produced about half the expected number of offspring.

Males appeared more affected by Klotho than females did. Their blood, unlike that of females, contained more insulin than normal mice. This suggested that the male mutants were somewhat resistant to insulin—a symptom, in extreme forms, of diabetes. The Klotho-boosted males and females had normal glucose levels, a surprise because untreated diabetes causes high glucose. These features don't appear in other long-lived mice, which are usually insulin-sensitive and have low glucose.

Klotho's effects on insulin could connect the protein to a hot story in aging research. Suppression of signaling by insulin and the related hormone insulin-like growth factor-1 (IGF-1) is one of the most consistently successful ways to extend life span in many species. Long-lived mice that are sensitive to insulin also usually have dampened insulin and IGF-1 signaling. ▶

PROTEOMICS

New Database to Track Protein Locations

Proteomics researchers in Sweden plan to release a database next week containing hundreds of thousands of images of where different proteins are located in human cells and tissues. The database, dubbed the Protein Atlas, is intended to help biochemists identify the function of newly discovered proteins. Although the new atlas currently contains data on only some 700 proteins, the Swedish team plans to tackle some 22,000 different proteins, one for each human gene.

"That's great," says Richard Smith, a proteomics expert at the Pacific Northwest National Laboratory in Richland, Washington. "It's one of the most valuable data sets you can have," adds Michael Snyder of Yale University, who pioneered a similar large-scale effort to localize proteins in yeast. The yeast data set, for example, has proven to be an essential tool in narrowing down whether proteins operate in the nucleus, the cell membrane, or elsewhere.

As scientists began to sequence human genes in the 1990s, sorting out the cellular locations of each gene's proteins became a priority, says Mathias Uhlén, microbiologist at the Royal Institute of Technology in Stockholm, Sweden, and director of the Protein Atlas effort. "This is something that has to be done to leverage the success of the human genome project," he explains.

A pilot project 2 years ago convinced the Knut and Alice Wallenberg Foundation of Sweden to bankroll a scaled-up Protein Atlas through September 2007. In April, it became one of six projects to be coordinated by the international Human Proteome Organisation (HUPO). Uhlén says he hopes HUPO member countries will finance the completion of the Atlas, which could take another 10 years.

To track down the location of proteins inside human tissues, Uhlén's team breaks the problem into two parts—finding antibodies that target individual proteins, and then using those antibodies to hunt for proteins inside tissues. To streamline this process, Uhlén's team has created standardized arrays containing microscopic tissue samples from 48 different normal human tissues and 20 types of cancer tissue. The antibodies are tagged so they can be seen and incubated with the arrays to reveal which proteins are expressed in each of the different tissues. The tissues are then photographed at high resolution, providing for each antibody hundreds of detailed images revealing where it has bound to its target protein. For now, Uhlén says, his team of about 100 scientists is creating half a dozen antibodies a day, leading to about 30 gigabytes of data for each antibody studied (which is stored at www.proteinatlas.com).

I spy. Glycoprotein SV2A (brown) in cerebellum tissue.

Today's arsenal of drugs, Uhlén notes, targets only 500 or so different proteins. By providing clues to the function of other proteins, he says, the Atlas may accelerate their use as markers for disease or drug targets.

The Protein Atlas still has wrinkles to be ironed out. If antibodies react with more than one protein, the tissue arrays may unwittingly spotlight unintended proteins. "There are huge issues of quality assurance," Uhlén says. As a result, his team will count on outside experts to flag problems. —ROBERT F. SERVICE

CREDITS (TOP TO BOTTOM): H. KUROSU; THE SWEDISH HUMAN PROTEOME RESOURCE

In rat cells, Klotho inhibited insulin signaling, making it tough for the hormone to do its job. Kuro-o's group also showed that some mice lacking Klotho survived somewhat longer and suffered fewer diseases when the team coaxed insulin and IGF-1 signaling back to normal. Klotho "ties in beautifully" with the IGF-1 story, says George Martin, a gerontologist at the University of Washington in Seattle.

Others are less sure. The link is "tenuous," says Luciano Rossetti, director of the diabetes research center at Albert Einstein College of Medicine in New York City. He points out that female mice with extra Klotho have normal insulin action but live substantially longer.

Kenyon says the new work raises the possibility that life span can be extended alongside mild insulin resistance, a trait considered deleterious to longevity. Researchers would now like to know if Klotho levels in humans correlate with life span—for example, if the blood of centenarians is swimming with it.

–JENNIFER COUZIN

ANIMAL BEHAVIOR

Tool Study Supports Chimp Culture

Chimpanzees may not have literature or ballet, but some researchers suspect that our close primate kin do have cultural traditions pertaining to behaviors such as tool use and grooming. Chimps in one forest might use a certain technique to scoop up tasty ants with a stick, for example, while those in another forest use a different method. But critics have argued that to qualify as culture, such local habits must be learned from fellow chimps—and that's been difficult to document in the wild.

Now, a study with captive chimps provides the first direct evidence that chimps can learn traditions of tool use by observation. "I think it's fantastic," says Carel van Schaik, a biological anthropologist at the University of Zürich in Switzerland who was not part of the research team. "This really nails down the social learning side of things." The authors of the study, published online 21 August in *Nature*, say their work also reveals another trait previously seen only in humans: a tendency to conform to community standards.

The view that chimps acquire the behavioral differences seen in the wild from imitating one another has been contentious (*Science*, 25 June 1999, p. 2070). The ideal field experiment—transplanting wild chimps from one population to another to see if they pick up new traits—is considered ethically untenable.

Instead, Andrew Whiten and Victoria Horner at the University of St. Andrews in Fife, U.K., and Frans de Waal at Yerkes National Primate Research Center in Atlanta, Georgia, selected a female of high social rank from each of two groups of 16 Yerkes chimps and gave the two private lessons on using a stick to obtain food from a specially designed dispenser. One female learned a "poke" technique; the other learned a "lift" technique. Back in their respective groups, each female's peers took notice of how she worked the dispenser, and the vast majority followed her example. Even when chimps stumbled on an alternative method, they tended to stick with what the rest of the group was doing, says Whiten.

CREDIT: DAVID BYGOTT

Culture shock. Chimp tool use reveals signs of culture.

The study "very convincingly mimics a situation that would happen in the wild," says van Schaik. One of the chimp culture skeptics, Bennett Galef, an animal behaviorist at McMaster University in Hamilton, Canada, says, "I've been looking for this [evidence] for 10 years."

Galef and others are less persuaded by the claim of social conformity. Van Schaik points out that chimps observe their group's favored technique more frequently, so their behavior could reflect what they've seen recently rather than a tendency to conform.

Although many researchers say the new study bolsters the case for chimp culture, others insist that chimps do not have the cultural sophistication of humans. Michael Tomasello, a comparative psychologist at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, suspects, based on recent work by his team, that the chimps in Whiten's study learned by watching the motions of the food dispenser rather than by imitating each other. Human culture is based very strongly on imitation, teaching, and language, he says. "What you have in chimps is different."

–GREG MILLER

ScienceScope

Europe Braces for Bird Flu

The European Union wants to keep the deadly H5N1 avian influenza strain out of European poultry flocks. Veterinary experts are due to meet this week, but several countries say they're not ready to follow Holland's drastic step of ordering all commercially raised birds indoors to prevent infection by migratory birds.

Outbreaks of H5N1 in Europe's vast poultry sector could have devastating economic effects, as has already happened in Southeast Asia. In the Netherlands—still reeling from a 2003 outbreak that decimated the industry—farmers were ordered this week to move birds inside or take other precautions to prevent them from mingling with wild birds. The measure, slated to last until the end of the fall migration at least, was relatively easy to implement because 95% of more than 100 million poultry in the country already live inside, says virologist Albert Osterhaus of Erasmus Medical Center in Rotterdam.

Germany is considering a similar move. But authorities in France and the United Kingdom—which have many more free-ranging birds than Holland does—aren't convinced migratory birds pose a great risk and say it's too early for such drastic measures.

–MARTIN ENSERINK

Crosshairs on Lung Cancer

Lung cancer kills more Americans than any other malignancy. But last year, the National Cancer Institute (NCI) spent more than twice as much on breast cancer—$566 million versus $277 million—not to mention $308 million on prostate cancer. To change that balance, NCI earlier this month announced it was committing up to $80 million more to enhance prevention and treatment of lung cancer as part of a businesslike initiative with strict milestones. The Bethesda, Maryland–based institute is searching for an outside director to oversee the initiative, which will span nicotine addiction, early detection of lung cancer, and drug development.

"It's certainly encouraging that they're beginning to think more about lung cancer," says Alan Sandler, medical director of thoracic oncology at Vanderbilt University in Nashville, Tennessee.

To drive home the call for research of all types, seven-time Tour de France winner Lance Armstrong lobbied President George W. Bush for a dramatic increase in cancer research funding during a 27-km bike ride at the president's Crawford, Texas, ranch last weekend.

–JENNIFER COUZIN

INFECTIOUS DISEASES

Global Fund Pulls Myanmar Grants

The Global Fund to Treat AIDS, Tuberculosis, and Malaria has canceled nearly $100 million of grants that over 5 years would have helped Myanmar fight the three diseases. Citing concerns about Myanmar's new restrictions on travel and procurement of medical supplies, The Global Fund announced on 19 August that it made the unprecedented decision to retract grants, saying the ambitious effort to prevent and treat these diseases "cannot be managed in a way that ensures effective program implementation."

Down and out. Myanmar relies heavily on international groups to provide services like this crowded AIDS hospice outside Yangon.

Myanmar receives scant international aid because of widespread distrust of the junta that runs the country, an impoverished Southeast Asian nation formerly known as Burma. The Global Fund, a Geneva-based nonprofit, thought it could prevent corruption by funneling money through the United Nations Development Programme, which would distribute the funds to nongovernmental organizations. The grants, awarded in April, also came with unusually stringent monitoring procedures. But last month, the junta announced new policies that nixed the deal, such as requiring 3-weeks' notice for any trips within the country, says The Global Fund spokesperson Jon Lidén. "You just can't run a program with conditions like that," says Lidén. "You can do something on a limited scale, but not at the pace our grants are expected to move."

One foreign aid worker in Myanmar who asked not to be identified says "political realities" doomed the program from the start. "As projects, they were overfunded and set unrealistic targets," he contends. Still, he urged other donors to "massively increase assistance" in a "more responsible package" that bolstered the private sector and selective government efforts.

However, one vocal critic of the junta, epidemiologist Chris Beyrer from Johns Hopkins University in Baltimore, Maryland, supported The Global Fund's approach and blames recent political turmoil within the junta for the program's demise. "It is just terrible for the people of Burma that the hardline faction of the junta now in power under General Than Shwe has again made it clear that political control remains so much more important to them than the well-being of the Burmese people," says Beyrer.

Although some in the U.S. government had initially expressed deep concerns about the grants to Myanmar, Lidén says no one from the Bush Administration or Congress pressured The Global Fund to scuttle the program. The Global Fund plans to wrap up all business by 1 December and recover much of the $11.8 million disbursed. **–JON COHEN**

NUCLEAR POWER

Ontario to Mothball Two CANDU Reactors

TORONTO—Only months after Canadian-made reactors were rejected in U.S. and Chinese markets, Canada's 60-year-old civilian nuclear industry has suffered a potentially mortal blow at home. Facing a $1.6-billion repair bill, the government of Ontario decided this month to mothball two 540-megawatt Canada Deuterium-Uranium (CANDU) nuclear reactors more than a decade before their projected retirement date.

"Ontario's decision to write off two reactors early could signal the end of the road for CANDU," says Tom Adams, executive director of Energy Probe, a nonprofit nuclear watchdog group based in Toronto. In January, the reactor company's U.S. partner, Dominion Resources of Richmond, Virginia, decided to abandon plans to seek a U.S. license for its next-generation CANDU. And in May, Chinese authorities announced that they weren't interested in buying any units beyond the two 700-megawatt units already operating near Shanghai.

Canadian officials have long touted the CANDU reactors, manufactured by the government-owned Atomic Energy of Canada Limited (AECL), as an example of the country's technological prowess. A descendant of the Manhattan project, CANDU's first forebear went on line at Chalk River, Ontario, in 1945. Since then some 34 large commercial versions have been built and installed around the world, including 20 in Ontario. But their complex cooling systems, which allow the reactor to be refueled without going off line, have proven very costly to maintain.

The reactors to be mothballed are two of eight at the Pickering Nuclear Station in the Toronto area. Built in the 1970s, they've been idle since 1997 largely because of thinning in the hundreds of pipes carrying heavy water coolant from the reactor core. Two years ago, three other laid-up Ontario reactors were restarted after refurbishments costing billions of dollars, and their operators now say more repairs are not far off. Adams says that CANDU reactors of various vintages in Argentina, India, Pakistan, Romania, China, and Korea will require extensive repairs sooner than planned.

Experts point to the corrosive effect of the heavy water coolant as a major culprit, with the reactor's design contributing to the large repair bills. "Just getting at the pipes is fantastically difficult, dangerous, and expensive," says Frank Greening, former head of nuclear cooling systems analysis at Ontario Power Generation (OPG), the government utility that owns all of Ontario's CANDUs. Even for reactors in which the coolant feeder pipes haven't yet deteriorated, says John Luxat, president of the Canadian Nuclear Society and OPG's former head of nuclear safety, "the costs of demonstrating [their safety] are becoming a problem."

Ken Petrunik, AECL's chief operating officer, says the CANDUs, which cost about $1.5 billion new, "perform well in their early years" and that their ability to refuel on line has yielded "better performance results than any other reactor type in the world." He downplays the impact of Ontario's decision to mothball two reactors by noting that AECL is only weeks away from launching a sales campaign for an advanced version of the CANDU reactor that will compete with new designs from other countries (*Science*, 19 August, p. 1168). Petrunik also discounted the recent bad news from the United States and China. "We remain confident we'll secure a reasonable share of the world market," says Petrunik.

–PAUL WEBSTER

Paul Webster is a freelance writer based in Toronto.

CREDIT: MALCOLM LINTON

GEOPHYSICS

Earth's Inner Core Is Running a Tad Faster Than the Rest of the Planet

The claim that Earth's inner core was getting ahead of itself seemed odd at first. Why should a 2440-kilometer solid iron ball spin faster than its 3000-kilometer-thick shell of mantle rock? Well, some computer simulations showed the molten-iron outer core dragging the inner core around by the magnetic field generated in the outer core. Still, seismologists had problems with measurements of the inner core's excess spin.

Now, 9 years later, the original claimants are back with persuasive evidence that the inner core really is spinning faster than the rest of the planet. Not as fast as it first seemed, but possibly fast enough to help probe the nature of Earth's layered interior.

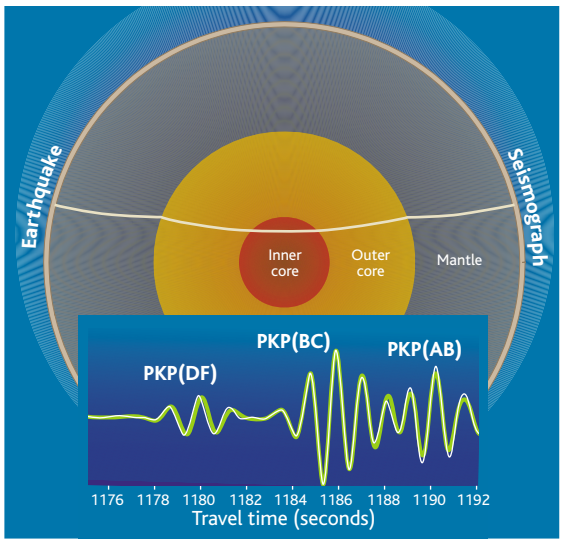
On page 1357, four seismologists—Jian Zhang and Paul Richards of Columbia University's Lamont-Doherty Earth Observatory in Palisades, New York, and Xiaodong Song and Yingchun Li of the University of Illinois, Urbana-Champaign—explain how they reduced the two sources of error bedeviling the original estimate of the inner core's rotation rate. One was the exact location of earthquakes near the South Sandwich Islands in the far South Atlantic Ocean. These moderate quakes send seismic waves down through the inner core and up to a seismograph in College, Alaska.

Thanks to a woodlike grain to the crystalline iron of the inner core, waves passing through it may slow down or speed up, depending on where they pass through. If the inner core rotates faster than the rest of the planet, quakes striking the same place years or decades apart will send out waves that take slightly different paths through the core. Waves from South Sandwich quakes would arrive in Alaska a little sooner than they did the time before, revealing the inner core's "superrotation."

Unfortunately, travel times to Alaska depend not only on the amount of inner core rotation but also on the quake's exact location. But seismologists can't tell precisely where such remote quakes are. So Zhang and colleagues hunted for a pair of quakes that have identical squiggles in their seismograms. For the wave shapes to match, the two quakes must take place less than a kilometer apart, says Song, and they probably overlap. Knowing that such doublets are so close to each other, the group could calculate that the travel time of the waves had changed 0.0090 second per year.

The other source of error is the uneven grain of the inner core. Nine years ago, this grain variation wasn't known, but Zhang and colleagues have mapped it using a technique introduced by seismologist Kenneth Creager of the University of Washington, Seattle. That information enabled them to calculate a superrotation of 0.3° to 0.5° per year, or about 900 years for the inner core to gain one full revolution on the rest of the planet. That's about a third as fast as Song and Richards's initial estimate of 1996 and a tenth of some later estimates. Seismologists are generally impressed. "This paper removes any lingering doubt as to whether the inner core is rotating at a different rate than the mantle," says Creager.

Researchers also seem to be homing in on the size of the excess rotation. Seismologist Guy Masters of Scripps Institution of Oceanography in La Jolla, California, has gauged inner core rotation at 0.1° per year, using an independent method that involves the quake-driven, bell-like ringing of the planet. "I'm happy with 0.2° [or] 0.3°" per year, he says, a range within the error of his estimate. Researchers can now consider what the observed superrotation says about Earth's interior or changes in the length of a day. It might help test computer simulations of how the outer core generates the magnetic field, says geophysicist Bruce Buffett of the University of Chicago, Illinois. That's a lot for a little extra spin. —RICHARD A. KERR



By a nose. Seismic waves from a later quake (white, *bottom*) follow the same path (*top*) but arrive later because the inner core has rotated more than the rest of the planet.

CREDIT: ADAPTED FROM ZHANG *ET AL.*, *SCIENCE*; ILLUSTRATION: PRESTON HUEY

ScienceScope

Higher Protection for Tiger Salamander

A U.S. District Court in California last week overturned a controversial decision by the U.S. Fish and Wildlife Service to downgrade protection for two populations of the California tiger salamander.

Tiger salamander populations in Santa Barbara and Sonoma counties were declared endangered in 2000 and 2003 respectively, a step that hampered developers. Last year, the agency downlisted the status of the populations to "threatened," explaining that the move would facilitate habitat conservation by ranchers (*Science*, 10 September 2004, p. 1554). Critics said the change was not scientifically justified, and last week, federal judge William Alsup agreed in his decision, calling the rule "riddled with error," and citing political meddling. The government might appeal the ruling, which reinstates the salamanders' status as endangered. —ERIK STOKSTAD

Spain Seeks Gender Equality in Lab

BARCELONA—The Higher Research Council (CSIC), Spain's main basic research agency, has announced a new policy to boost the proportion of women researchers. About a third of the 2369 scientists supported by the CSIC are women, which follows the European Union average. But just 15% of lab directors and other top positions are female. To push the total share of women researchers to 40% or more, women scientists will now make up at least 40% of selection boards tasked with appointing new CSIC scientists. The move follows a March initiative by the government to promote gender equity in Spanish society.

Former CSIC scientist Maria Blasco, a telomerase expert at the Spanish National Cancer Center in Madrid, said that the 40% goal was a good start but that a "commitment" was needed to have more women in top research roles.

—XAVIER BOSCH

Dr. Frist Prescribes ID

Senate majority leader Bill Frist (R–TN) cheered scientists last month with his unexpected support for embryonic stem cell research. But last week, he disappointed many when he told reporters that students should be taught Intelligent Design (ID) as well as evolution. "I think today a pluralistic society should have access to a broad range of fact ... including faith," he told the Associated Press. Teaching both evolution and ID "doesn't force any particular theory on anyone." —CONSTANCE HOLDEN

Using construction cranes to reach above towering treetops, scientists are achieving a better overview of forest ecology and how trees contribute to global climate change

Sky-High Experiments

Plant ecologist Christian Körner of the University of Basel, Switzerland, goes to work by soaring into the sky on a construction crane. He and his colleagues squeeze into a four-person cage and, in 30 seconds, are carried up 30 meters. The crane operator guides the gondola to the end of the 45-meter-long boom and slowly lowers it, leaving Körner and his colleagues dangling just above the 30-meter-tall treetops of the Swiss forest they're studying.

Körner's first ride more than a decade ago was an eyeopener. "The canopy was not the green carpet we thought, but highly structured, with peaks, gullies, canyons, and deep gorges among some crowns," he recalls.

Once a novelty, cranes have become essential for sorting out forest dynamics, say ecologists. Most of a tree's photosynthesis occurs in its canopy—the upper leaves, twigs, and branches—and 40% of the world's terrestrial species live there. From their lofty perches on cranes, researchers have been counting species and studying leaf and tree physiology for more than a decade. More are now turning their attention to global change. Körner, for example, wants to know how forests capture greenhouse gases. On page 1360, he and his colleagues report findings from the first phase of a long-term experiment looking at carbon dioxide's effects in established forests. "[This study] is our first real glimpse of how mature forests might respond to increasing concentration of atmospheric carbon dioxide," says Kurt Pregitzer, an ecologist at Michigan Technological University in Houghton.

Körner is among several hundred ecologists, plant physiologists, taxonomists, and conservationists who have moved their studies off the forest floor to the more productive upper layers. These researchers work at about a dozen crane sites scattered around the world (see map, p. 1315). But if they can cobble together a relatively modest amount of money, these researchers have even more ambitious plans. In an effort called the Global Canopy Program (GCP), Körner and his colleagues are pushing to double the number of research cranes and train more students, scientists, community leaders, and educators in their use.

Lift off. Körner (*inset*) and his team do their work dangling 30 meters above the ground.

From the top

Linking the earth and sky, canopies harvest energy from the sun and create organic matter. They provide moist and dry spots, as well as warm and cold pockets, making possible a huge diversity of forest fauna. Canopies also play a role in global climate change, although researchers have yet to pin down exactly how. For example, trees suck in carbon dioxide for use during photosynthesis, whereas microbes release it by degrading fallen canopy leaves.

Although forest researchers are often willing to don climbing equipment to scale tree trunks or build walkways that sway among the branches, these strategies afford only a partial view of the canopy. The tops of trees either can't be reached from below or can't support the weight of people. In contrast, cranes offer a top-down perspective that forest researchers have wanted. In the past 15 years, "cranes have become the symbol of canopy research," says Kamal Bawa, head of the Ashoka Trust for Research in Ecology and the Environment in Bangalore, India.

In 1992, Alan Smith of the Smithsonian Tropical Research Institute (STRI) in Panama was the first to get this bird's-eye view of a canopy, using a 40-meter-high crane set up among the trees in a Panama City park. The vista was breathtaking and the view of the greenery below, stupendous. By swinging the crane's boom around in a circle and shuttling the gondola along its length and lowering the cage to different heights, researchers could finally get the big picture of a canopy.

A second crane was set up in 1997 in a different spot in Panama, a site where some 85 ecologists and taxonomists are now using a range of techniques designed to pin down the number and identities of arthropod species in the canopy. Established in 2003, the arthropod project now has 400,000 specimens and 1080 species in its archives. As it continues, researchers expect to find many thousands more specimens and large numbers of new species. Only with this many samples "can the many patterns of diversity, community organization, and functional roles of individual taxa [in the canopy] be understood," says forest ecologist Andreas Floren of the University of Würzburg, Germany.

CREDITS: CHRISTIAN KÖRNER

Once Panama's cranes began proving their worth—typically the investment requires several hundred thousand dollars per site—other groups began procuring cranes for temperate sites. In 1999, Körner used a helicopter to deposit a crane in a century-old Swiss woodland, whose trees tower 30 or more meters above the ground. Despite the importance of biodiversity studies, Körner took another tack with his crane. "A logical next step [was] getting involved in the larger process studies," including experiments related to greenhouse effects, he says.

Until Körner's project, those studying the forest effects of increased carbon dioxide had limited their attention to young trees—no taller than 16 meters and primarily in single-species plantations of sweet gum or loblolly pine. In these younger forests, ecologists pumped carbon dioxide from towers to blanket the young trees. However, they could not apply this technique to taller, more mature trees.

Körner overcame this drawback by placing 10 kilometers of drip irrigation tubing among the upper branches of a 500-square-meter plot. His team pumped carbon dioxide through the tubing, delivering 50% more than ambient concentrations to each tree. "My prime intention was to break the technological barrier that so far limited research to young, vigorously growing trees," Körner explains.

The carbon dioxide pumped through the tubing incorporated more than the usual amount of an unusual carbon isotope, distinguishing it from the gas absorbed normally from the atmosphere. In this way, Rolf Siegwolf and Sonja Keel of the Paul Scherrer Institute in Villigen, Switzerland, were able to track the fate of the extra carbon as it cycled through the forest ecosystem. At the same time, Körner's graduate student Roman Asshoff monitored tree growth. "This is certainly a much more realistic approach than studying potted plants or young trees in plantations," says Yves Basset, an STRI entomologist.

By focusing on mature trees and extending measurements to the ground, Körner was able to assess tree-soil interactions. Whereas young trees use extra carbon to speed up growth, mature ones don't, he and his colleagues report in this week's issue of *Science*. Instead, much of this carbon winds up in the roots, ultimately moving into the soil, where microscopic fungi take up much of it. Thanks to microbial activity, "this carbon is rapidly recycled to the atmosphere through the root zone," says Körner.

Different species of trees processed the extra carbon differently, but some trends were clear. Overall, carbon in the soil increased by 44%. Furthermore, the makeup of decomposing leaves changed. Lignin, a polymer that combines with cellulose to stiffen trees, dropped by 11%, whereas the amount of starches and sugars increased by 14%. As a result, decomposition sped up. The results highlight the critical connection between the canopy and the ground, says Pregitzer.

More labs with a view

Körner now wants to help carry out larger-scale experiments with several cranes and to replicate the carbon dioxide work around the world in different forest types. About a decade ago, fellow forest ecologists created the International Canopy Network, which now includes more than 750 researchers from 62 countries. In the mid-1990s, the U.S. National Science Foundation (NSF) funded a canopy-research database that has fostered better collection, storage, analytical, and visualization techniques, including three-dimensional representations of the data.

The 4-year-old GCP, which is complementary to the International Canopy Network, is building on this momentum. It hopes to develop a more global view of biodiversity and climate change effects by doubling the number of existing cranes. Most of the new cranes proposed by GCP would be erected in tropical forests. Brazil, Ghana, Madagascar, India, and Malaysia have already signed on to host these so-called whole forest observatories.

The key, of course, is finding the money. Over the past decade, only about $4.5 million a year has been spent on canopy work worldwide. Coming up with $17 million over the next 5 years would pay for five of the 10 new observatories called for by GCP. The goal is to have the whole program up and running by 2020. In March, the United Nations Environment Program endorsed GCP's proposal, although to date it has only given GCP $30,000.

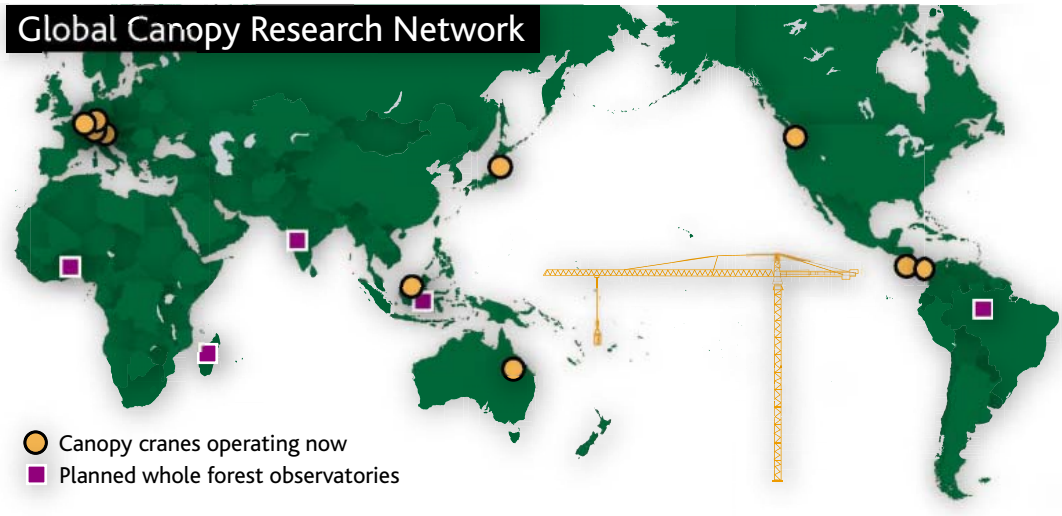
To qualify for the next level of United Nations support—about $500,000 for designing the sites—GCP must come up with $1 million. The five countries tabbed for whole-forest observatories have promised to help fund infrastructure and some of the research. The rest of the money must come from funding agencies of other governments or private foundations, says GCP head Andrew Mitchell.

The only U.S.-based crane canopy site, in Wind River, Washington, is supported by the National Forest Service (NSF). NSF also provides grants to individual canopy scientists, who pay a "bucket fee" of $185 a day. And the U.S. Department of Energy has a big project on climate change at the Wind River site.

The uncertain financial picture for GCP's plan isn't preventing some hosts of the new whole-forest observatories from forging ahead. The Indian government has provided the Ashoka Trust with seed money to start a canopy program in western India, and last week, researchers held a planning meeting. Canopy researchers elsewhere are tweaking their activities to conform with the whole-forest observatories protocols.

Thanks to these efforts, "the focus on canopy research will change from the more-or-less isolated investigations to globally coordinated projects with comparable methods," says Martin Unterseher of the University of Leipzig, Germany. This integration is essential, he adds, if scientists hope to ever understand the relationship between forest biodiversity and global change.

—ELIZABETH PENNISI



Lofty goal. Placing cranes in new places will fill out the network of existing crane research plots and lead to better integration of canopy studies.

SOURCE: GLOBAL CANOPY PROGRAM

New French Agency Tries Out 'Anglo-Saxon Style' Reviews

French researchers are debating the pros and cons of having a National Science Foundation of their own

PARIS—"Une petite revolution" is how one French newspaper recently described the new National Research Agency (ANR) that in October will start handing out money to research groups across the country. Its modus operandi—selecting research projects based on scientific excellence—is standard elsewhere in the world. But in France, where funds are traditionally given in block grants to institutions and labs and then distributed to individuals, and where being a scientist often means having a lifetime government job, the notion is revolutionary.

It's also controversial. Many researchers worry that ANR, with a starting annual budget of €350 million ($420 million) that's set to grow rapidly, will eventually cannibalize vaunted government strongholds of French science such as the much larger National Center for Scientific Research (CNRS) and the similarly sized National Institute for Health and Medical Research (INSERM). Moreover, some say that the agency—modeled on long-established outfits like the U.S. National Science Foundation and the German Research Foundation—introduces a type of personal competition that simply isn't right for France. "We have a different organization," says Edouard Brézin, president of the French Academy of Sciences. "One shouldn't simply copy models from abroad without thinking." Even researchers who welcome the idea of spicing up research with a bit of competition fear that ANR, operating with a minuscule staff and zero tradition, won't measure up to the quality standards of the foreign examples it seeks to emulate.

Those concerns don't seem to bother the agency's director, Gilles Bloch. What counts, says the 44-year-old biophysicist and physician, is that the research community has responded overwhelmingly. With almost all of ANR's first 35 calls for proposals now closed, some 5300 applications have poured in on topics such as biotechnology and CO_2 capture and storage. More than 600 researchers volunteered to be reviewers. About a quarter of the proposals will receive awards. ANR, Bloch says, "is clearly going to be an important new factor in French science."

Creative tension? ANR director Gilles Bloch hopes to give French science a competitive edge.

CREDIT: *LE FIGARO*

Grand strategy

ANR, whose goal is to make research more dynamic, promote excellence, and give young people more opportunities, is part of a larger plan that's still in the works. In February, as debate flared up around a major reform bill, the government decided to go ahead and create the new agency under a temporary legal structure. Researchers are still waiting to see the bill, now promised for the fall (*Science*, 11 February, p. 829).

The concept isn't really a break with tradition, Bloch insists: ANR takes the place of two funds, now dissolved, which doled out money on a project-by-project basis: the National Fund for Science and the Fund for Technological Research. They reported directly to the ministry of research, however, and both were widely suspected of being subservient to politics. Besides having a much more generous budget, ANR will be autonomous in selecting grantees.

Bloch says he looked closely at examples in the United States, the United Kingdom, and Germany in planning ANR, which he joined early this year after 3 years at a high-level job at the research ministry. But the French agency has some key differences. Scientists receiving ANR money will have to be on the payroll of one of the research institutes or universities, for instance. ANR grants—some half a million euros on average—can be used to help hire a postdoc or technician and pay for instruments or supplies, but they don't pay a researcher's salary. In addition, ANR staff, currently just 30, will be kept well below 100 and will take care only of overall management and quality control. Running the funding programs, including the peer-review process, will be contracted out to research organizations and universities.

Some researchers doubt whether such a small, central organization can judge so much science. The frantic handling of the first wave of proposals—necessary because rules dictated that the initial budget, resulting from privatizations, had to be spent this year—doesn't bode well, says cell biologist Bruno Goud of the Curie Institute in Paris. A member of one of ANR's scientific councils, he had to help recruit reviewers for stacks of proposals submitted in the large "nonthematic" program. "It was pretty messy," says Goud. (Finding someone to review a proposal about the sexual life of oysters on short notice was a particular challenge, he recalls.) Still, the agency is a step in the right direction, he emphasizes: "Maybe it will work better next year."

Others have been less charitable. Brézin, who co-chaired a committee last year that organized a 6-month national debate about the future of French science, says he and many others in the research community "were never opposed to the principle" of awards based on merit. But the government seems intent on using the agency as a way to attack established research agencies such as CNRS and INSERM, he says.

Many agree that these flagships of French science can be overly bureaucratic and unwelcoming to new ideas, and that it takes too long before young researchers are allowed to form their own research groups. (Former research minister Claude Allègre recently called them "Soviet-style" institutes.) Last fall, scientists reached a consensus at a meeting in Grenoble for stricter evaluations, fewer rules, and more money, among other reforms. Creating a large new agency, however, was not on the list, says Brézin.

Brézin and others also fear that ANR may soon outgrow the other funding agencies: The government has promised a budget hike of €240 million next year, or

68%, and a copy of the reform bill leaked in January pegged ANR's budget at almost €1.5 billion—and its effect would be multiplied because it doesn't have to pay researchers' salaries. If this comes to pass, "we will have one giant and a lot of dwarfs," says Alain Trautmann, the public face of Sauvons la Recherche, a protest movement that brought thousands of researchers onto the streets last year to protest cutbacks in research funding. Trautmann worries that the Anglo-Saxon–style focus on individual competition will put researchers under enormous pressure and isn't convinced that it will lead to more creativity.

Bloch, who worked as a visiting scientist at Yale University in the early 1990s, says he admires the dynamism of American science but isn't a fan of the stress it creates, either. The French situation, he notes, is very different: Job security isn't at stake here, and INSERM and CNRS aren't under siege. But he believes that the country's scientists must learn to compete more at home if they want to remain competitive internationally. "We can stay as we are," he says, "and say that the rest of the world should be more like France. But that won't help us."

–MARTIN ENSERINK

Archaeology

Maya Archaeologists Turn to the Living to Help Save the Dead

To preserve ancient sites, pioneering archaeologists are trying to improve the lives of the Maya people now living near the ruins

Archaeologist Jonathan Kaplan tries to spend as much time as possible exploring Chocolá, a huge Maya site in southern Guatemala dating from 1200 B.C.E. So far his team has mapped more than 60 mounds, identified dozens of monuments, and found signs of the emergence of Maya civilization, including large, sophisticated waterworks that likely required social organization to build.

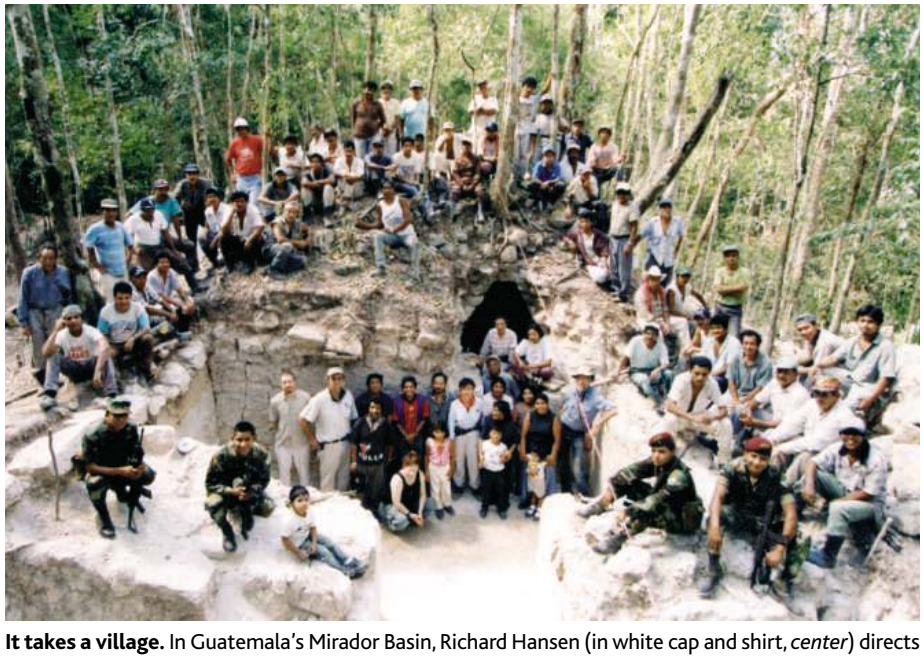
But today, instead of digging, Kaplan is lunching with the mayor of a municipality that includes the impoverished town of Chocolá. Kaplan, a research associate with the Museum of New Mexico's Office of Archaeological Studies in Santa Fe, is trying to enlist the mayor's support for a land swap that would give farmers land of no archaeological value in exchange for land that holds Maya ruins. The local people he's trying to help, many of them descended from the ancient Maya, are "clinging by their fingers to survival," says Kaplan. So, working with a Guatemalan archaeologist, he has established a trash-removal service, hired an environmental scientist to help improve the drinking water, and developed plans for two museums to attract tourists.

Kaplan and others are in the vanguard of a movement called community archaeology. From Africa to Uzbekistan, researchers are trying to boost local people's quality of life in order to preserve the relics of their ancestors. In the Maya region, the situation is urgent; the vestiges of the ancient Maya may be destroyed in 5 to 10 years unless something is done to curb looting, logging, poaching, and oil exploration, says Richard Hansen, president of the Foundation for Anthropological Research & Environmental Studies and an archaeologist at Idaho State University in Pocatello. Hansen, Kaplan, and others are using archaeology as an engine for development, driving associated tourism and education projects. The resultant intertwining of research and development is such that "I cannot accomplish the one without the other," says Kaplan, "because poverty is preventing the people from attending to the ancient remains in a responsible fashion."

It wasn't always that way. Until fairly recently, Maya researchers were solely focused on the hunt for "stones and bones," says Hansen. Archaeologist Arthur Demarest of Vanderbilt University in Nashville, Tennessee, says researchers often excavated a site with the help of local workers, only to abandon them when the project ended. Those who lost their income often resorted to looting and slash-and-burn agriculture to survive. "In the wake of every archaeological project is an economic and social disaster," says Demarest.

He offers one of his own projects as an example of what not to do. After employing about 300 people in the early 1990s at several sites in the Petén, the vast tropical forest in northern Guatemala, Demarest left the government with a continuing development plan for the region, much of it federal land. But the federal government brought in outsiders to implement it. Desperate at having lost their jobs, the local people plundered the sites.

"From that, I learned a lot of lessons," Demarest says. "Archaeology transforms a region." In his view, archaeologists themselves must take responsibility for helping the locals succeed. "The days of Indiana Jones, when archaeologists could go to a place, excavate, and then leave without concern about the impact that their actions are having on the people in the area, are gone," he has said.



CREDIT: CHARLES DAVID BIEBER

It takes a village. In Guatemala's Mirador Basin, Richard Hansen (in white cap and shirt, *center*) directs scores of trained local workers in restoring an ancient Maya city.

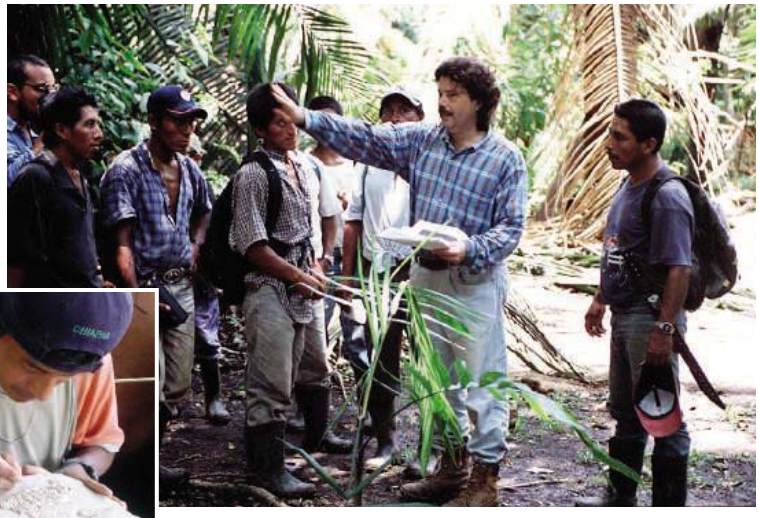
Today, Demarest embraces this responsibility as he excavates part of the great trade route that ran through much of the Maya region, including along the Pasión River and through Cancuen, an ancient city in central Guatemala. He says his project is successful because it operates "bottom up—we're working through the village." Using ethnographic studies of the Maya people and working with leaders from several villages, Demarest designed a research and community development plan that enables the local people, rather than outsiders, to serve as custodians of their own heritage. The communities choose projects—archaeology, restoration, ecotourism, etc.—and run them with the guidance of experts, earning more than they would by farming.

One successful enterprise is a boat service, run by the Maya, that ferries tourists down the Pasión River from the village of La Union to Cancuen, now a national park. In addition to generating revenue, the service attracted a variety of agencies that provided potable water, electricity, and school improvements to La Union. The World Bank cited the boat service as one of the 10 most innovative rural development projects in the world in 2003.

Demarest also helped establish a visitor center, an inn, a guide service, and a campground at the park's entrance. Three nearby villages collaboratively manage these operations, and the profits pay for water systems, school expansions, and medical supplies. "The only way these things are going to succeed is if it's theirs," says Demarest, who has raised nearly $5 million for community development at Cancuen. Last year, he became the first U.S. citizen to be awarded the National Order of Cultural Patrimony by the Guatemalan government.

Other archaeologists are trying to achieve similar results in their own field areas. Hansen is exploring the origins, the cultural and ecological dynamics, and the collapse of the Preclassic Maya (circa 2000 B.C.E. to 250 C.E.) in the Mirador Basin. His project has a budget of $1.2 million, with about $400,000 going to development and $800,000 to archaeology. He raised roughly half of the funds from the Global Heritage Fund, a nonprofit organization that helps preserve cultural heritage sites in developing countries. The project employs more than 200 people who earn above-average wages while getting training; Hansen's team has also installed a new water system and bought 40 computers to boost locals' computer skills.

Looting in the basin has been devastating in the past, so Hansen has hired 27 guards—most of them former looters. They make good guards, he says, "because they know the tricks of the trade." The project has instilled "a sense of identity" in some residents, although Hansen acknowledges that others continue to loot. "It is a long battle to win the hearts and minds of these people," he says.



Learning to lead. Guatemalans trained by Arthur Demarest (*above, center*) lead tours and carve stone miniatures of ancient monuments (*inset*).

Although both Demarest and Hansen have won generous grants for their work, they agree that finding funding for community archaeology is "horrific," as Hansen puts it. Kaplan makes do with about $130,000 each year for his "terribly underfunded" project, although his ideal would be about $800,000. Traditional funders, such as the U.S. National Science Foundation (NSF), pay for research but not community development, says Demarest. NSF, with its modest budget of $5 million to $6 million, is most interested in the "intellectual merit" of a project, agrees archaeology program director John Yellen, although he adds that the foundation does consider "broader impacts," including community development. Demarest, who is financed by some 20 organizations including the United States Agency for International Development and the Solar Foundation, says a big budget is a must for community projects: "You've got to have about $400,000 a season to do ethical archaeology."

But other researchers say it's possible to run such projects without big budgets. Archaeologist Anabel Ford of the University of California, Santa Barbara, who has been practicing small-scale community archaeology while studying land-use patterns at a large site called El Pilar on the Belize-Guatemala border since 1983, says that she can achieve her community development goals for as little as $12,000 a year. "I actually think it's not about tons of money," she says. "It's about consistency."

Ford operates on an annual budget of $30,000 to $75,000, with funding sources ranging from the Ford and MacArthur Foundations to her own pocket. Within El Pilar's lush tropical forest are numerous temples and other buildings that stand as high as 22 meters. Over the years, Ford has built a cultural center and a caretaker house, and El Pilar now attracts hundreds of ecotourists annually. Ford started an annual festival to celebrate cultural traditions and foster community involvement, and she's organizing a women's collective to sell local crafts. "We've built the first infrastructure at El Pilar since 1000 [C.E.]," she says.

Whether they operate with big money or on the cheap, community archaeologists face a delicate juggling act between development and research. Ford believes her academic career has suffered because of the time and effort she's invested in development projects. "I would have written much more substantive work on my research at El Pilar," she says, lamenting that she has yet to finish a book about her work. Kaplan and Demarest say that they spend about half their time on community development, leaving only half for archaeology.

As impressive and well-intentioned as these and other community archaeology projects seem, at least a few researchers are concerned about unintended consequences. "If you don't understand the local politics, you can really do damage," says Arlen Chase of the University of Central Florida in Orlando, who has investigated Caracol, a major Maya site in Belize, since 1984. It's difficult to determine just what archaeologists owe the community they work in, he adds. "This is a new endeavor, and we're learning how best to do it," agrees archaeologist Anne Pyburn, outgoing chair of the Ethics Committee of the American Anthropological Association.

Despite these concerns, Hansen and his colleagues seem convinced that they're making progress. Guatemalans who were "dedicated to looting and destroying these sites," Hansen says, are "now dedicated to preserving them." —MICHAEL BAWAYA

Michael Bawaya is the editor of *American Archaeology*.

CREDITS: ARTHUR DEMAREST

A 'Robin Hood' Declares War on Lucrative U.S. Patents

A 30-year-old former corporate lawyer says that the U.S. patent system leaves the public with the short end of the stick

"Did Pfizer get punked by a nonprofit?" That's what patent lawyer and blogger Stephen Albainy-Jenei asked in June after the U.S. Patent and Trademark Office (PTO) ruled that a Pfizer patent for Lipitor, the $12-billion-a-year cholesterol drug, might be invalid.

The decision was the latest in a string of successful initial rulings for Dan Ravicher, a 30-year-old attorney and crusader against those patents that he says are bad for the public welfare. He's also used PTO procedures to shoot holes in patents held by Microsoft and Columbia University. Part vigilante, part gadfly, Ravicher has quickly earned a reputation for being part of a new breed of patent attorneys, and one worth watching.

"The system has been created in a way that makes it difficult to see how it impacts people," Ravicher says. He believes patent busting could result in cheaper and better consumer products by removing barriers to innovation by the public, which he feels is left out of the equation. He hopes his efforts will inspire others to challenge the system by drawing attention to bad patents.

Ravicher works through the Public Patent Foundation (PubPat), a nonprofit organization he created 2 years ago. Its actions have already received the attention of intellectual-property insiders. Hal Wegner of Milwaukee, Wisconsin–based Foley and Lardner calls him a "Robin Hood" for the patent world's have-nots. "What he's doing is important," says healthcare analyst Les Funtleyder of Miller Tabak, a New York brokerage firm. "Nobody's really kept an eye on what pharma's doing from a patent perspective."

His corporate opponents won't comment on their plucky new adversary. But critics say the current patent system serves the U.S. economy well by rewarding innovation. They also warn that Ravicher's efforts could backfire by making it harder for makers of low-cost generic drugs to get their products to market.

Ravicher didn't start out planning to be a burr in the side of corporate America. After graduating from the University of South Florida with a degree in materials science and then the University of Virginia School of Law, Ravicher became a New York patent attorney whose clients included the drug giant Johnson & Johnson. But as he watched small IT companies wage expensive battles against what seemed to him bad patents, he became convinced that the current system "more often than not treated the less-represented unfairly." By living frugally off his six-figure income and winning a small foundation grant, he managed to put together $90,000 to start the foundation. He's still on a tight budget: Only by persuading his landlord to reduce the rent were he and his girlfriend able to hang on to their Manhattan apartment.

As the foundation's executive director and only full-time employee, Ravicher supervises a handful of volunteer scientists, occasional grad students, and legal interns as they search for potential flaws in big-name patents. He targets them because he believes they "are causing the most harm." For example, he says, Pfizer's patent on Lipitor, in force until 2017,

precludes other companies from developing "a safer, less side-effect–causing Lipitor." Spurious software patents, he adds, reduce competition and drive up prices.

Ravicher's tool of choice is PTO's reexamination request system. He claims three recent successes—"three for three [attempts]," crows PubPat director Eben Moglen of Columbia Law School—support his argument that PTO issues extremely lucrative patents based on ideas already in the public domain. His Columbia challenge involved a 2002 patent for the gene-inserting process called cotransformation used in making drugs. The university's fourth such patent, the technology has netted the school hundreds of millions of dollars.

Ravicher argued that all subsequent claims were identical to the school's 1980 patent. In that patent, he wrote, Columbia had described a process for "generating … DNA molecules" that was identical to a claim in the 2002 request for a way of "producing the proteinaceous material." Both would result in replicated DNA and translated proteins, he notes. (Facing lawsuits, Columbia later agreed not to assert the patent.)

In 2003, Microsoft sought to license a file-storage system called FAT, crucial to the operation of Windows. Months later, Ravicher filed a reexamination request on the company's 1996 patent, pointing to two prior software patents that he said rendered the patent obvious. Neither one had been mentioned in paperwork by the examiner who granted Microsoft's patent. (The company says its patents' file system goes beyond its predecessors; after PTO issued its initial approval, attorneys hailed Ravicher's move in the trade press.)

"The more technology becomes a part of life, the more likely the patent system's failings are going to affect daily life."

–Dan Ravicher

Last year, Pfizer used its 1999 patent (one of five involving Lipitor) to sue a series of Web sites selling a generic version, atorvastatin, made in Canada. Ravicher argued that the 1999 patent—for the crystalline form of the drug—was obvious in light of two previous Pfizer patents. PTO agreed, arguing that both were in fact crystalline atorvastatin, challenging Pfizer to show otherwise. Last week, Pfizer told PTO the previous forms of the molecule were amorphous, not crystalline, despite having used the word "recrystalized" to describe the process.

The stakes are high: If successful with final rulings, Ravicher's moves could cost Microsoft millions in licensing revenue and bolster a campaign against Columbia's blockbuster patents. Investors expect generics to defeat the Lipitor patent before its 2017 expiration, says pharmaceutical analyst Jon LeCroy of Natexis Bleichroeder in New York. But Ravicher wants to speed up their progress.

CREDIT: MARTY KATZ

PubPat's method has been taken up by the Electronic Frontier Foundation in San Francisco, California, and the Washington, D.C.–based Patients Not Profits is using similar tactics to scrutinize drug and software patents. But not everybody agrees with Ravicher's approach. Skeptics note that lawsuits, although more costly, are much more effective than reexaminations, in which patentees may argue back and forth with examiners and challengers are excluded. For its part, PTO resents the implication that it doesn't represent the public's interest. And attorney Steven Lee of Kenyon & Kenyon in New York City says reexamination requests such as Ravicher's can "screw it up" for other patent challengers, including makers of generic drugs, if the government reaffirms the validity of the patent. Ravicher has a simple answer to that last charge: Bad patents pollute the system, he says, and generics merely seek duopolies.

Ravicher knows he's fighting an uphill battle. But he says that events such as the 2001 anthrax letter attacks, which spawned a debate over whether the government should break Bayer's patent on Cipro to prepare for bioterrorism, illustrate the flaws of the system. "The more technology becomes a part of life, the more likely the patent system's failings are going to affect daily life," he says.

–ELI KINTISCH

Meeting Earth System Processes 2

Major Shifts in Climate and Life May Rest on Feats of Clay

CALGARY, ALBERTA—From 8 to 11 August, an interdisciplinary meeting organized by the Geological Society of America and the Geological Association of Canada covered topics from life's origins to the future climate.

When scientists look at the climates of the past, hydrology gets short shrift. What the atmosphere was actually made of—how much carbon dioxide, how much methane, how much oxygen—is the subject of heated debate. How much rain fell out of it is not. But perhaps it should be. Various researchers here explored the possibility that past hydrology matters a great deal because rain makes clays, and clays can play a crucial role in the carbon cycle. Clays, according to Martin Kennedy of the University of California, Riverside, represent "the most intimate relation of the mineralogical and the biological [parts of the earth system]"—and one he thinks has been badly overlooked.

Take the Paleocene-Eocene Thermal Maximum, a sudden climate shift 55 million years ago that has recently become a hot topic (*Science*, 28 February 1997, p. 1267). Billions of tons of carbon, probably in the form of methane, were somehow released into the atmosphere in a geological instant, raising the global temperature by as much as 8°C and radically reshaping the carbon cycle in the oceans. The total amount of carbon that poured into the atmosphere seems to have been similar to that which would be released if humanity burns its way through all the currently accessible fossil fuels.

Gabriel Bowen of the University of Utah in Salt Lake City has been looking at where all that carbon ended up. Although various measurements suggested that plants on land, encouraged by a climate that was suddenly not just warmer but also a lot wetter, had mopped up the lion's share of the stuff, there was no evidence that the carbon was permanently stored on the continents. Instead, Bowen is exploring the idea that carbon bearing the isotopic signature of land plants ended up in fine particles stuck to clay minerals buried in sediments on the continental shelves. The increased rainfall was creating more clays than usual. Those clays not only helped carbon move from the continents to the seas, but they also protected the carbon when it got there, by shielding it from the predations of organisms.

Clear as mud? Clays washed from land into offshore waters could have altered the carbon cycle, with huge impacts on atmosphere and climate.

Kennedy, a former petroleum geologist, thinks clays also had a crucial effect on a much earlier chapter in Earth's history: the rise of oxygen shortly before the first animals emerged some 600 million years ago. Some researchers think that such a change in the composition of the atmosphere helped make complex life possible (*Science*, 17 June, p. 1730).

Clays, Kennedy argued in one talk, tend to form much more easily in soils where living organisms are around to help break down rock minerals. So before life reached the continents, the rate of clay production would have been far lower, and with it the capacity for clay-assisted carbon burial in shallow seas. After lichens colonized the land, the rate at which clays were formed by the rain and washed into the sea would have risen, boosting the burial of organic carbon offshore. Normally, creatures living in the ocean would have combined that carbon with oxygen from photosynthetic organisms, turning it into CO_2. With more carbon buried out of harm's way, Kennedy argues, excess oxygen was free to escape into the atmosphere. Kennedy acknowledges that there is only very limited evidence for lichen at the time, and land plants didn't arise until millions of years later. But he says a variety of circumstantial evidence suggests that the continents were getting more weathered around then.

Kennedy and Bowen are plowing new furrows in their field. Although soil scientists take for granted the key role clay plays in carbon burial today, most geologists studying the fossil record have yet to apply that lesson to the past. "We have only just realized that we have to think about [the role of clays]," says Thomas Wagner, who presented a paper on swings in the carbon cycle that caused the oceans to lose their oxygen during the Cretaceous period. Wagner has just left the University of Bremen, Germany, to join a soil science group at the University of Newcastle-upon-Tyne, U.K., hoping to adapt methods

CREDIT: CORBIS

used to study contemporary soils for his geological work. "We're really at the beginning of something," he says.

Kennedy agrees—and thinks the change in the way geologists see sedimentary carbon burial and the connections between continents, continental shelves, and rainfall may challenge current ideas throughout the geological record. "When the pendulum swings, it will swing heavily," he predicts.

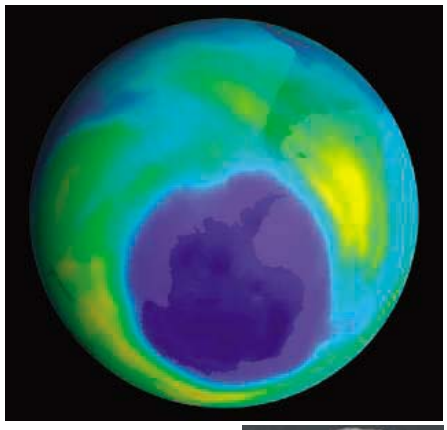
Specks of Evidence For Ancient Sunburn

The ozone hole that has afflicted high southern latitudes for the past couple of decades has little to recommend it. But it's been a useful calibration device for Barry Lomax and colleagues at the University of Sheffield, U.K. Ozone depletion may have played a role in various past extinctions, but ozone doesn't leave much of a fossil record. Now, by studying spores from club mosses on South Georgia, a small British island east and a little south of the Falklands, Lomax thinks he may have found a way to tease out fossil ozone levels.

Spores and pollen need to protect their DNA while they blow around the world. Some plants impregnate the particles' coats with pigments that absorb ultraviolet light, especially DNA-damaging UV-B. Lomax told the Calgary meeting that the level of these pigments in South Georgia spores had increased as the ozone hole had deepened over the years. In the tropics, samples of the same species showed no change in protective pigment over the same time period—but the levels increased in mosses that grew at higher altitudes, where the UV is more intense.

Using this benchmark, Lomax and his colleagues hope to find evidence for ozone depletion in ancient spores that still bear the chemical traces of these pigments. To start with, they are studying the Permian period, at the end of which Earth suffered its greatest mass extinction. Oxygen levels are thought to have dropped considerably over the Permian, and fossil pollen studies may show whether ozone followed suit.

Lee Kump, an earth scientist at Pennsylvania State University, University Park, has a theory that predicts massive ozone loss at the end of the Permian, so it's no surprise he was excited by the prospect of a new technique that might back up his ideas. But "there's no shortage of ways to destroy ozone at the end of the Permian," he admits. "The guy sitting next to me had one, too." Hydrogen sulfide escaping from an anoxic sea (Kump's choice), vast outbursts of methane (his neighbor's), ozone-destroying chemicals made by volcanoes, or even an asteroid or cometary impact could all have done in the ozone layer.



Connect the dots. Club-moss spores (*right*) may show whether today's hole in the ozone layer had a Permian precursor.

David Beerling, a professor of paleoclimatology at Sheffield who has overseen Lomax's research, says the Permian is an ideal test case to start with: "It's the big one, because the signal is so strong and there are a lot of terrestrial [rock] sequences" with spores in them. If the technique works out, he hopes to extend it to other periods to see if there's a "bigger picture" in the history of ozone depletion throughout the time that plants have been around to record its effects.

Storms Bow Out, But Boughs Remember

For a tree battered by its gusts, a hurricane is nothing but trouble. But it's just a welcome late summer downpour for those out of harm's way. That downpour carries with it an intriguing isotopic marker that seems to make tree rings a better repository of the hurricane record than meteorological measurements or historical records.

Claudia Mora, a geochemist at the University of Tennessee, Knoxville, is leading an interdisciplinary study of the isotope markers that hurricanes leave in tree rings. All evaporation and precipitation cycles have an effect on the oxygen isotopes in water, a process known as Rayleigh distillation. But in hurricanes the effect is particularly striking, with rainwater strongly depleted in oxygen-18. In plants with shallow roots, such as longleaf pine, this isotopic signature gets quickly incorporated into wood.

Mora and her colleagues have made detailed studies of the isotopes in the tree rings of longleaf pines from Lake Louise in Georgia. Over the past century, they found a strong oxygen-isotope signal in the wood laid down in the latter part of growing seasons marked by hurricanes. By studying dead trees preserved in water and swamps, they have extended the record back several centuries.

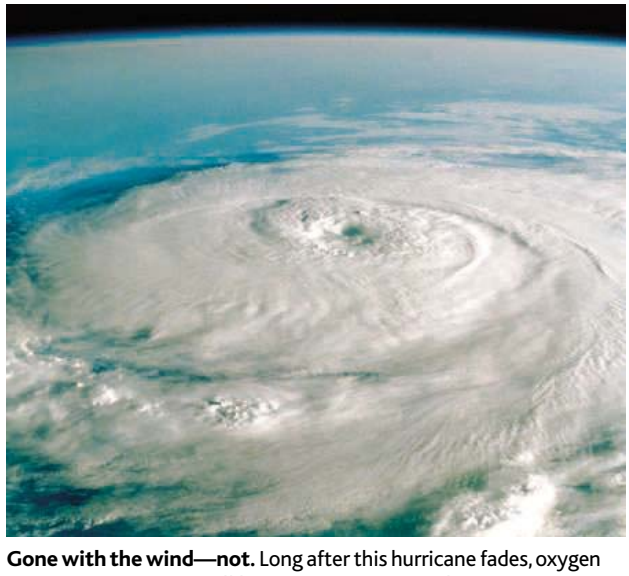
Tree rings from the 18th century showed what seems to be the first mainland evidence of the "Great Hurricane of 1780" that ravaged Cuba. Mora also found 40 years in the late 16th and early 17th centuries with "no evidence of a single hurricane impacting the isotopic balance." That coincides with a previously studied period of intense drought in the African Sahel. Hurricane frequency and Sahel rainfall are correlated, so that 40-year hurricane-free patch looks very plausible—and the technique quite robust.

The challenge now is to extend the isotope technique to other sites and to tie it to other factors influencing hurricanes. The data seem to show ways of distinguishing different sorts of hurricanes, and also to tease apart different phases of climate oscillations that seem likely to be linked to the conditions that give rise to hurricanes, such as the Atlantic Multidecadal Oscillation (*Science*, 1 July, p. 41).

Climatologists "are still trying to work out what the decadal and multidecadal controls on hurricane frequency are," Mora says. But even a century-long instrumental record may not be enough, she adds: "What we're trying to do is give them 500 years [in which] to see patterns."

–OLIVER MORTON

Oliver Morton is a writer based in the U.K.



Gone with the wind—not. Long after this hurricane fades, oxygen isotopes in tree rings will bear witness to its might.

CREDITS (TOP TO BOTTOM): NASA; (INSET) BARRY LOMAX AND CHARLES WELLMAN; PHOTOS.COM

Random Samples

Edited by Constance Holden

Bees for Van Gogh

Bee inspects blue signature on Van Gogh painting.

An ecologist and an artist have collaborated in a fanciful project exploring bees' response to paintings of flowers.

Behavioral ecologist Lars Chittka of the University of London and artist Julian Walker designed an experiment that involved showing bumblebees several famous paintings, one of them Van Gogh's *Sunflowers*, in order to "provoke thinking" about differences in visual perception between bees and humans and the reasons the two species are attracted to flowers.

Whereas people see three basic colors—red, yellow, and blue—bees see blue, green, and ultraviolet. The researchers put a nest of bees that had never been exposed to flowers in a lab together with four paintings and then counted how many times the bees approached or landed on them. Van Gogh's *Sunflowers* proved most attractive. Of 146 approaches, 17 were to the blue "Vincent" signature. The painting also got the most landings: 15, compared with four each for two colorful nonflower paintings. A preference for blue was seen in all the bee landings, presumably because blue flowers "offer high-nectar rewards," the authors reported online last week in the journal *Optics & Laser Technology*.

The study is "consistent with what is currently known about bee physiology and behavior," says vision researcher Adrian Dyer of La Trobe University in Victoria, Australia. "Bees do have innate color preferences for blue flowers and for spatial features that are flowerlike."

Money Can Buy (Some) Happiness

"Men do not desire merely to be rich, but to be richer than other men." So said philosopher John Stuart Mill about 150 years ago. Now sociologists are chiming in with a study showing that money buys happiness—as long as it puts people ahead of their peers.

Many surveys have shown that more money doesn't necessarily translate into more happiness. Glenn Firebaugh, a sociologist at Pennsylvania State University, University Park, and Harvard grad student Laura Tach devised a method to try to zero in on the relationship. Mining 30 years of survey data on well-being, they sorted some 20,000 working-age Americans by income and then by whether they thought themselves as "very," "pretty," or "not too" happy. The data also covered age, health, marital status, education, race, work status, and gender, so the researchers were able to compare individuals with otherwise similar profiles.

Firebaugh and Tach concluded that money makes people happiest when they have more of it than those in their bracket. But it's not as important as health or marriage, they reported last week at the annual meeting of the American Sociological Association in Philadelphia. Economist Richard Easterlin, who studies income and happiness at the University of Southern California in Los Angeles, calls the study a "thorough, painstaking analysis."

Video Wars

The rocketing popularity of computer games has ratcheted the video-violence debate up to new levels. Last week, the American Psychological Association (APA) in Washington, D.C., adopted a resolution calling on makers of video games for youth to reduce violence levels. "A review of research shows that playing violent video games can heighten aggression," APA trumpeted in a press release.

However, the first long-term study of online video game playing fails to support that premise. Conducted by Dmitri Williams, a speech communication professor at the University of Illinois, Urbana-Champaign, the study involved 75 people of both sexes, mostly young adults, who spent 56 hours over the course of a month playing "Asheron's Call 2"—a game with lots of fantasy violence—in their homes. Players as well as 138 control subjects had their attitudes and argumentative behaviors tested before and after the trial. There were "no strong effects associated with aggression caused by this violent game," says Williams. "Given that the finding was opposite the APA's predictions, I think this should remind us how little we know about this medium." The study appeared in the June issue of *Communication Monographs*.

Yale psychologist Dorothy Singer, a member of the group that proposed the APA resolution, believes the results of short-term

laboratory studies, which indicate temporary increased aggressiveness after gameplaying, are convincing. They indicate "an effect size the same as smoking and cancer," she says. "This has to be taken seriously."

100 Gigabases and Counting

This week, the number of nucleotide bases in the world's three major databases—at the European Bioinformatics Institute (EBI) in the U.K., the DNA Data Bank of Japan, and GenBank—topped 100 billion, about equal to the number of nerve cells in a human brain, a National Institutes of Health press release points out.

The three databases share data every night, enabling scientists to instantly assess whether a DNA sequence has already been discovered. There's just one problem, says EBI bioinformaticist Ewan Birney. The databases, which have been doubling in size every 14 months, "are still growing faster than our computing capacity."

CREDITS (TOP TO BOTTOM): INSET: COPY BY JULIAN WALKER; TIBURON INC.

Edited by Yudhijit Bhattacharjee

CAMPAIGNS

Groundswell. A new grassroots lobby group, modeled on the Democrats' Moveon.org, has joined the stem cell advocacy landscape. StemPAC was launched last month by 38-year-old John Hlinko, who worked on Wesley Clark's failed attempt to win the Democratic presidential nomination in 2004. So far, there's one scientist among the group's advisers: neural stem cell researcher Evan Snyder of the Burnham Institute in La Jolla, California. "We're just starting to reach out to other scientists," says Hlinko.

Hlinko says he's not worried that his partisan background might alienate some members of his target audience, pointing out that support for the cause comes from both sides of the aisle. He claims that the group has already had an impact by preparing a TV ad scolding Senate Majority Leader and presidential hopeful Bill Frist (R–TN) for failing to allow a vote on legislation to expand the number of stem cell lines available to federally funded researchers. He thinks that the ad, which would have run in New Hampshire, home of the first presidential primary, played a role in convincing Frist to back the legislation (*Science*, 5 August, p. 858).

DEATHS

Star figure. Astrophysicist John Bahcall, whose idea of studying the sun by measuring the number of solar neutrinos reaching Earth paved the way for fundamental discoveries in astrophysics and particle physics, died from a rare blood disorder in New York City on 17 August. A professor at the Institute for Advanced Study (IAS) in Princeton, New Jersey, and former president of the American Astronomical Society, Bahcall was 70.

In 1964, Bahcall and Raymond Davis Jr. laid the foundations for neutrino astrophysics by proposing that the number of neutrinos reaching Earth could shed light on the sun's characteristics. Experimental observations by Davis later showed a discrepancy between Bahcall's predictions and the number of neutrinos detected, which kicked off a 3-decade effort to solve the "solar neutrino puzzle." The answer was that neutrinos have mass and switch between different particle states.

"Always generous with his time, John Bahcall was an inspirational teacher and mentor who shaped the careers of a generation of scientists," says IAS Director Peter Goddard.

MONEY MATTERS

Profit sharing. A microbiologist has pledged at least $105 million to New York University (NYU) School of Medicine from royalties for a blockbuster drug that he helped invent.

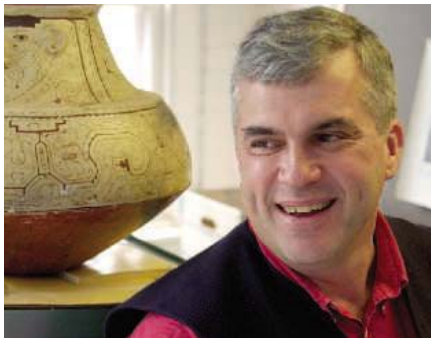
Jan Vilcek, 72, says the school took a chance when it hired him 40 years ago, after he and his wife fled Communist-ruled Czechoslovakia using a weekend pass to Vienna. In the late 1980s, Vilcek led a team at NYU in developing a novel antibody that became the basis for Remicade, which treats arthritis and Crohn's disease.

NYU has already received a portion of the gift from Vilcek, who has been receiving royalties since Centocor began selling the drug in 1998. Another portion will flow to the school in quarterly payments tied to sales, which last year reached nearly $2 billion. The money from the gift, to be paid over 13 years, will support new faculty, fellowships for graduate students, and research equipment.

MISFORTUNES

Amazon tragedy. An American archaeologist and anthropologist whose research suggested that pre-Columbian people in the Amazon practiced sustainable development and conservation was killed on 13 August during a research trip to the region.

James Petersen, chair of anthropology at the University of Vermont in Burlington, was shot to death during a restaurant robbery in a small rainforest town in Brazil where he had been doing fieldwork. Police are holding three suspects, according to an Associated Press report.



Petersen's South American research was revolutionary, challenging a long-held belief that the Amazonian environment couldn't sustain complex societies, says University of Vermont anthropologist John Crock. His wide-ranging fieldwork also included sites in the Caribbean and in northeastern New England.

Petersen "wasn't Indiana Jones, out for fame and fortune," says anthropologist Michael Heckenberger, a former student now at the University of Florida, Gainesville. "You couldn't ask for a better colleague, mentor, or friend." He was 51.

EXPLORERS

Cold sweat. Three Polish researchers narrowly escaped from an approaching pack of hungry polar bears last week in the Arctic, according to an Associated Press report. The men had set out from the Polish research ship *Horyzont* in a small inflatable boat to pick up equipment from one of the islands in Norway's Svalbard Archipelago, about 1000 km from the North Pole. When their boat capsized in rough seas, they swam to the island of Edgeoya. The trio started a fire and kept the bears at bay for several hours until rescue helicopters arrived.

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CREDITS (TOP TO BOTTOM): JOANNE SAVIO; JOHN HLINKO; IAS; UNIVERSITY OF VERMONT

LETTERS

Sacrificing Dialogue for Politics?

THE RECENT STATEMENT OF VIENNESE Cardinal Schönborn "clarifying" the Catholic position on evolution is disconcerting (*1*). Schönborn, who is a close ally of Pope Benedict XVI, declares that "evolution in the neo-Darwinian sense is not true" and that there is "overwhelming evidence for design in biology," thus aligning the Catholic Church with the Intelligent Design movement.

The strategy is familiar. The sophistication of evolutionary theory is misrepresented, and the process is cartooned as solely consisting of random mutation and natural selection, thus concocting a facile state of disbelief in the audience. Ample reference is made to Church documents, which declare that an unguided process of evolution outside the bounds of divine providence "simply cannot exist." In this medieval logic, the existence of divine intentional design seems inevitable.

Many processes in nature appear to be guided, such as a stretched rubber band becoming as short as it can despite none of its many parts "knowing" ahead of time what that configuration is. Similarly, the evolutionary process rests on the dynamics of molecular and developmental interactions that collectively shape the outcomes of random mutation and selection in a nonrandom way. This weaving together of evolution and developmental processes provides the modern experimental and theoretical framework, grounded in Darwinian thinking, for explaining the organization of living systems.

Unlike a dogmatic Church, science offers an iterative method of observation and reason that has proven to be mankind's most fruitful approach to truth. Cardinal Schönborn brands the scores of researchers that follow the scientific method of inquiry as ideologues, while proclaiming the Church as the "firm defender of reason." This sounds like Galilei all over again, if it wasn't for this last surreal move, which represents a sweeping attack on science in general at a time when so many domains of western society structurally depend on it. Herein we discern some intent that goes well beyond ignorance of scientific facts.

Schönborn's statement shows how fragile the relations between science and religion still are and how tempting it is to sacrifice dialogue for politics. The Catholic Church—indeed, any major religion—should be a partner in much-needed reflections about the societal implications of science. As Austrian evolutionary biologists, we stand against the statements expressed by the Austrian Cardinal and shall continue a dialogue with those who are not bent on fundamentalism.

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> "Schönborn's statement shows how fragile the relations between science and religion still are and how tempting it is to sacrifice dialogue for politics."
>
> —*LAUBICHLER* ET AL.

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Paradigm Shifts Needed for World Fisheries

THE POLICY FORUM "ECOSYSTEM-BASED fishery management" by E. K. Pikitch *et al.* (16 July 2004, p. 346) proposes ecosystem-based fishery management (EBFM) as a new direction for fishery management, reversing the order of priorities, to start with ecosystem considerations rather than the target species. EBFM has been recommended as a holistic management approach, mainly to solve industrial fishery problems (bycatch, habitat perturbation, etc.), by U.S. advisory panels (*1–3*). These recommendations largely ignore artisanal (small-scale) fisheries, which involve more than 50 million fishers around the world (*4*), a number constantly increasing because of high unemployment rates, poverty, and food scarcity. Industrial and artisanal fisheries cannot be lumped together, as they operate on different scales and require different management solutions. For industrial fisheries, the top short-term management priorities are (i) reduction of fleet, ground facilities, and subsidies; (ii) moratoria on new entrants into the business; and (iii) administration of catch quotas (*5*, *6*). In artisanal fisheries, the implementation of these tools is unrealistic, because of the large social and economic costs for developing countries and because there is not sufficient information about local ecosystems (*7–9*). Therefore, management would mainly be based on precautionary approaches. Alternatively, societal incentives (e.g., territorial user rights for fishers, co-management, and community quotas) have been shown to solve artisanal fishery problems, where due respect to traditions is a key driver (*7*, *10*).

The world fishery crisis is a series of complex, multifaceted problems, embedded in different societies. New perspectives for rational management require paradigm shifts, including EBFM, but principally incentives for effective governance and sharing of management roles between government and local organizations (*7*). In our view, legitimizing the participation of fishers in the planning and surveillance of management measures is a promising short-term solution to current artisanal fishery crises, promoting compliance with regulations (*7*, *11*).

Ocean zoning [e.g., marine protected areas (MPAs)] has also been suggested as a critical element for EBFM. This will be difficult to achieve in industrial and artisanal fisheries, due to high enforcement costs. Implementation of MPA zoning cannot be considered as a short-term solution to current fishery crises. Although we welcome EBFM and marine protected area approaches, we feel that there is a risk associated with overemphasizing them. Management options must be also used according to societal and cultural backgrounds. Sound fishery science is a necessary, but not sufficient, condition for the sustainability of marine resources.

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International Gaps in Science Publications

THE POLICY FORUM "INCREASING INTERNATIONAL gaps in health-related publications" by G. Paraje *et al.* (13 May, p. 959) shows clearly that the large majority of biomedical research is carried out in high-income countries. The authors do not make clear, however, that these countries comprise a small percentage of the world population (15%) and account for an even smaller share of the global disease burden (*1*, *2*). The consequence is that the overall research portfolio of the world is inevitably severely distorted in favor of the diseases of the rich, such as cancer and heart disease, and against those of the poor, notably HIV/AIDS, malaria, and tuberculosis. But it also means that from the perspective of the rich countries, their major diseases can actually be underresearched, as cancer is in Europe (*3*) (particularly compared with the situation in the United States). Meanwhile, malaria turns out to be over-researched, relative to its burden, in all but two of the 14 World Health Organization world regions, the exceptions being southern Africa and some countries in the eastern Mediterranean region (this is notably so in the UK, which accounts for nearly 20% of all relevant papers but suffers little directly from the disease), but globally underresearched by a factor of about eight, with less than $7 spent per disability-adjusted life year compared with $60 on cardiovascular disease and over $100 on diabetes (*4*). This distortion is yet another serious consequence of the unequal division of health-related research between the countries and regions of the world.

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THE STIMULATING POLICY FORUM BY G. Paraje *et al.* "Increasing international gaps in health-related publications" (13 May, p. 959) was widely discussed at my institution in Bangladesh. The observation of the widening gap in numbers of scientific publications between high- and low-income nations is not surprising, given the fewer numbers of scientists in the low-income countries and the constraints they face.

The authors correctly point to "brain drain" from low- to high-income countries. From my experience in Bangladesh, well-qualified local scientists generally prefer to remain in their home country if they can find meaningful employment in institutions where they can be productive.

Well-functioning institutions contribute to "brain gain," thus increasing the scientific and economic resources of a country as a whole. Ideally, these institutions in low-income countries should be connected to the international scientific community through Internet access, access to literature, and partnerships with international scientists from other institutions.

Unfortunately, many donor organizations have shifted toward "targeted project funding" rather than institution building. Many donors provide minimal or no indirect costs, and few are interested in funding capital items or buildings. Even fewer will contribute to endowments for institutions in developing countries, yet they give generously to these same items in the United States.

Building institutions where low-income country scientists can be productive is not easy, and there is no single successful model. Some countries like Korea and China have decided to support such institutions themselves. Most low-income countries do not have sufficient financial resources to do this, but they can facilitate the establishment of such institutions and can encourage donors to contribute, as has occurred in Bangladesh. More effort is needed to understand the factors that enhance sustainability of successful research institutions in low-income countries.

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Response

OUR ANALYSIS PROVIDED FURTHER EVIDENCE that scientific publications on a broad range of health topics, not just biomedical sciences, are disproportionately distributed and highly concentrated among the world's richest countries as well as within each economic category of countries, and that the gap in the output between low-income countries and the rest of the world widened between 1992 and 2001. Indeed, as Lewison and others (*1*, *2*) have underlined, a major disequilibrium exists among countries and regions between research funding, capacity, output, and dissemination and burden of disease and population, referred to as the "10/90 gap" (*3*). Research priorities do and should reflect a range of social and scientific values, not only disease burden. But the magnitude and persistence of the "10/90 gap" clearly calls for a change in the way that priorities and investments in health research are made around the world involving policy processes, research infrastructure development, and social debate. Whether an optimal solution exists remains unclear. Would, for example, a 50/50 equilibrium ensure effective and efficient use of resources to improve health around the world?

Nevertheless, to move toward a more representative research enterprise and one that is eventually relevant to the majority of the world's population, research capacities need to be greatly enhanced, particularly in low-income countries. Sack's Letter highlights strong institutions as a key ingredient to this issue and raises the challenge that institution building requires partnerships among many actors who are jointly interested in sustained and longer term development.

Along these lines and in collaboration with networks of policy-makers and researchers in 13 low- and middle-income countries involved in the Health Research Systems Analysis Initiative of the World Health Organization, we have collectively identified key factors that contribute to an enabling environment for those managing, conducting, and disseminating research addressing health topics (*4*). These include: (i) range and breadth of research networks; (ii) transparency of the funding process; (iii) quality of work space and facilities; (iv) encouragement of collaboration; (v) opportunities to present, discuss, and publish results, including scientific journals, media, and national and international conferences; (vi) addressing priorities that are relevant at national or international levels; (vii) adequate salary and benefits to recruit and retain trained professionals; (viii) career nurturing; (ix) training and ongoing training; and (x) access and sharing of information.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

On the basis of surveys in these 13 countries, we found, surprisingly, that better remuneration was not among the top priorities for improvement from the perspective of those working in these countries. Rather, transparency of the funding process, quality of work spaces and facilities, and training and ongoing training were consistently ranked as the most important areas for further strengthening. Similarly, financial investments alone, without changing attitudes and behaviors, are unlikely to yield results. All of these measures are related to well-functioning institutions and, more broadly, to a stable macro-environment.

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1. R. Horton, *Lancet* **361**, 712 (2003).
2. "The 10/90 Report on Health Research 1999" (Global Forum for Health Research, Geneva, 1999).
3. Made well known by the Global Forum for Health Research, this slogan initially describes the disequilibrium between global spending on health research and the diseases or conditions that account for global disease burden. The "10/90 Gap" has now been infused with many similar interpretations, capturing a range of inequities across high-income countries and the rest of the world.
4. World Health Organization (WHO), "World report on knowledge for better health: strengthening health systems" (WHO, Geneva, 2004), p. 82, box 3.8.

CORRECTIONS AND CLARIFICATIONS

Reports: "Spectral signatures of hydrated proton vibrations in water clusters" by J. M. Headrick *et al.* (17 June, p. 1765). The authors wish to acknowledge the pioneering contribution of H. A. Schwarz [H. A. Schwarz, *J. Chem. Phys.* **67**, 5525 (1977)] for his first report of the vibrational spectra displayed by small protonated water clusters. In particular, his identification of the strong 2660 cm^{-1} band with the $H_9O_4^+$ Eigen ion was confirmed in a size-selective study by Okumura *et al.* [M. Okumura, L. I. Yeh, J. D. Myers, Y. T. Lee, *J. Phys. Chem.* **94**, 3416 (1990)], and the authors' recent work supports their assignment of this band to the asymmetric stretch of the embedded H_3O^+ ion.

Perspectives: "Air pollution–related illness: effects of particles" by A. Nel (6 May, p. 804). In the right-hand panel of the figure on page 804, the scale bar should be 40 nm, not 40 μm.

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Slip-Rate Measurements on the Karakorum Fault May Imply Secular Variations in Fault Motion"

Erik T. Brown, Peter Molnar, Didier L. Bourlès

Mid-Pleistocene slip rates derived from cosmic-ray exposure ages for moraines offset by slip on the Karakorum Fault exceed modern values, a finding that led Chevalier *et al.* (Reports, 21 January 2005, p. 411) to hypothesize secular variation in fault movement. A more conventional interpretation of these widely scattered ages indicates lower slip rates and eliminates arguments for temporal variability in rates.
Full text at www.sciencemag.org/cgi/content/full/309/5739/1326b

RESPONSE TO COMMENT ON "Slip-Rate Measurements on the Karakorum Fault May Imply Secular Variations in Fault Motion"

M-L. Chevalier, F. J. Ryerson, P. Tapponnier, R. C. Finkel, J. Van Der Woerd, Li Haibing, Liu Qing

Correlation between surface exposure age clusters on the Manikala moraines and local/global temperature minima supports deposition during MIS 6 and 3-2, implying negligible surface degradation. Because it is improbable that the older moraine was emplaced before MIS 6, the slip rate on the Karakorum fault must be greater than 9 mm/yr.
Full text at www.sciencemag.org/cgi/content/full/309/5739/1326c

OCEANOGRAPHY

From the Beach to the Sea Floor

Alistair Sponsel

Fathoming the Ocean
The Discovery and Exploration of the Deep Sea
by Helen M. Rozwadowski
Harvard University Press, Cambridge, MA, 2005. 288 pp. $25.95, £16.95. ISBN 0-674-01691-2.

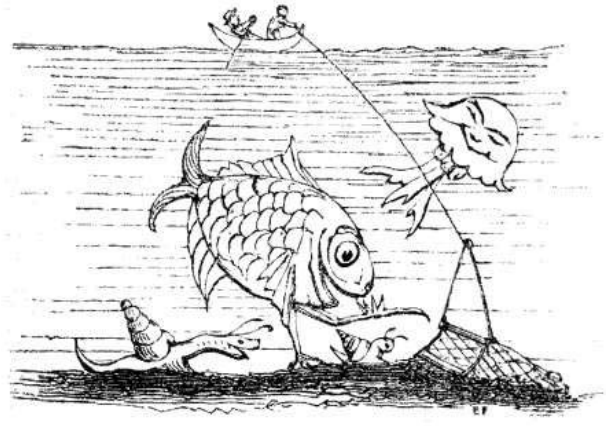
Just 200 years ago the nature of the deep sea and the location of the ocean floor—if indeed there was one—were not merely unknown but largely unimagined. As Helen Rozwadowski points out in *Fathoming the Ocean*, at the end of the 18th century even explorers viewed the sea as a void: the purpose of setting sail was to reach land again as soon as possible. In this illuminating study, she argues that the deep ocean was only "discovered" as an object of scientific study in the middle of the 19th century, when Western attitudes about the ocean shifted away from understanding it "as an expansive divide, a watery highway, or an unfathomable barrier between places," toward "a cultural redefinition of the sea as a destination and a location."

Rozwadowski, a historian of science at the University of Connecticut at Avery Point who has previously written on the international politics of ocean science and management, now delves into the 19th-century origins of oceanography. She observes that cultural recognition and scientific study of the deep ocean emerged rapidly together between 1840 and 1880, when the sea became a more familiar part of everyday life in Britain and the United States through the rise of commercial transatlantic shipping, the flourishing of maritime literature, and the Victorian beachcombing and aquarium crazes. However, as she notes, the most important factor prompting the mid-century discovery of the deep ocean was the immense government and private investment in efforts during the 1850s and 1860s to lay a submarine telegraph cable across the Atlantic. Cable surveys produced a wealth of new knowledge of the ocean floor, and telegraph boosters used the resulting sounding data and bottom samples to usher away old perceptions of the deep sea as a forbidding wilderness. They urged the public to envision, in Rozwadowski's words, "a cable resting safely and peacefully in a previously unimaginable place."

One of the most impressive elements of the book is the author's depiction of the equipment and techniques that, beginning in the late 1840s, British and American hydrographers used to sound unprecedented depths. Mariners, and even naval surveyors, were previously concerned primarily with ensuring that the sea was deep enough for navigation, a purpose served adequately by "no bottom" measurements. Rozwadowski shows that pioneering efforts to sound to the deep ocean floor required immense physical effort and were often more valuable as experiments in the development of sounding practices and equipment than as sources of reliable data. Hauling in 1000 fathoms of line required (by one account) the work of 100 men for an hour and 20 minutes. One sounding from the 1850 voyage of the U.S. Coast Survey's schooner *Taney* played out 5700 fathoms of line. Keeping the line "dead up and down" and determining when, if ever, the sounding lead had reached the sea floor were the greatest technical challenges of early blue-water hydrography. The *Taney*'s record sounding was among many early results that were discredited in the next decade, notably by measurements made using John Brooke's 1853 sounding device that released its sinker upon striking the bottom.

Naturalists were the second group that "brought [the sea] into focus as an object of scientific inquiry," although their conception of "deep water" lagged well behind the hydrographers' until the 1860s. Like hydrographers, they "began close to shore, probing blindly…into ever deeper water." Marine naturalists adapted the dredge from fisheries, and many dredging enthusiasts became "cruising yachtsmen" in the pursuit of zoological novelties. Although dredging flourished in local natural history societies on both sides of the Atlantic, several prominent British naturalist-dredgers moved to London around 1860 seeking the application of metropolitan resources to the study of life in the deepest parts of the ocean. When the Royal Society successfully petitioned the Admiralty to make navy vessels available for study of the ocean floor, the captains of H.M.S. *Lightning* and H.M.S. *Porcupine* modified tackle that had been recently developed for deep-sea sounding to allow dredging at depths approaching a thousand fathoms. Although Rozwadowski argues that it was "novel only in its huge scale," the British *Challenger* expedition (1872–76) was the technological and organizational culmination of the new scientific fascination with the sea. Its circumnavigation of the globe resulted in a 50-volume report (published 1880–1895) renowned as the foundation of 20th-century research in ocean zoology, geology, chemistry, and physics.

Historians of science may read *Fathoming the Ocean* most enthusiastically as a contribution to the geography of scientific knowledge. A rapidly growing body of historical literature seeks to demonstrate the influence of physical locations (such as labs, museums, field sites, or specific institutions or nations) on the practices and prestige of scientists. Rozwadowski's work is well poised to catch this historiographical wave, because the book addresses the importance of scientific "places" at a number of levels. Besides treating the distinct Anglo-American national contexts, Rozwadowski explains how physical and zoological studies of the ocean fit among other field sciences in a period that also saw the rise of professional geology and a surge of astronomical expeditions and polar explorations. She follows 19th-century marine scientists as they moved out from the shoreline on rowboats, pleasure craft, and finally working oceanographic vessels, enabled by a shift of patronage in the opposite direction, from local societies in coastal towns to elite metropolitan institutions. In doing so, she also provides a striking counterpoint to studies of lab-



"Odd fishes to entangle!" Edward Forbes's enthusiasm for natural-history dredging led him to pen "The Dredging Song" (1840), in which this line appears, and this cartoon from his *Natural History of the European Seas* (John van Voorst, London, 1859).

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oratory life by demonstrating how the succession of specific shipboard working spaces shaped the social and material cultures of the emerging discipline of oceanography.

The book's considerable appeal is enhanced by the inclusion of several dozen outstanding illustrations, and Rozwadowski's prose conjures further images of early ocean scientists' battles with hemp, brine, and benthic ooze. The narrative is occasionally repetitious as it jumps back and forth between Britain and the United States, and readers may also be left to wonder whether ocean science developed similarly in other countries. Nevertheless, *Fathoming the Ocean* will clearly be welcomed as a serious contribution by historians of science, technology, and maritime culture. And in addition, as the foreword by marine biologist Sylvia Earle underscores, the story is also of immediate relevance to anyone who wonders when and how we came to understand—as we now urgently do—the ocean's importance to our blue planet.

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ENVIRONMENT

Too Wed to the Sea

The Science of Saving Venice
by Caroline Fletcher and Jane da Mosto

Umberto Allemandi, Turin, Italy, 2004. 92 pp. Paper, $14.50, £7.99. ISBN 88-422-1310-1.

Venice is a city whose strength and vulnerability have always depended on the sea. From a tiny settlement founded in the fifth century A.D., it grew to become the heart of a dominant maritime power by the 14th century—an ascendancy due in large part to its location within a lagoon on the edge of the Adriatic Sea. Venice's famous fleet of commercial ships could easily embark on their immensely profitable voyages, while the city remained protected from enemies by water too shallow for attack by sea and too deep for attack by land. It is ironic then that the very setting that allowed Venice to flourish is the one that puts it in such danger.

The magnitude of this threat became apparent on 4 November 1966, when the city was submerged by a disastrous storm surge that raised the local water level by nearly two meters, flooding more than 90% of the city. Since that time, the protection of the city from such floods has been the subject of vigorous investigation and debate. In *The Science of Saving Venice*, Caroline Fletcher and Jane da Mosto summarize what is known about the problem and some potential solutions. Their account is distilled from the efforts of the U.K.-based Venice in Peril Fund and its Venice partner, CORILA (the Consortium for the Coordination of Research Activities Concerning the Venice Lagoon System).

Venice has always had to endure floods, as the city's highest point rises only two meters above sea level. But the frequency and intensity of *acqua alta* (literally, "high water") events have increased dramatically over the past century. (In the early 20th century, St. Mark's Square flooded less than 10 times a year; now that number is more than 60.) The increases are due to a combination of factors including the natural subsidence of the region, a 50-year period of accelerated subsidence in the middle of the 20th century caused by excessive extraction of groundwater (which was stopped in the early 1970s), and a gradual deepening of the lagoon as a result of sediment starvation and removal by increasingly strong tides. Together these factors have made the city more prone to inundation by the water piled up in the northern Adriatic by storms, which sometimes overwhelms the natural and man-made barriers protecting Venice. If current trends continue, the city eventually will be all but uninhabitable and beyond restoration.

How, then, can Venice be saved? The consensus solution, a focus of the book, relies on erecting across the lagoon's three inlets a series of mobile flood barriers that can be raised whenever large storm surges are expected. Construction has already begun on this project, the Modulo Sperimentale Elettromeccanico (called MOSE, like the biblical patriarch). The giant gates of these barriers are intended to protect Venice from the highest floods for at least the near future, but their longer-term success is threatened by both natural subsidence and the sea-level rise anticipated from global warming. As the authors recognize, the task of preventing Venice from flooding is not merely an engineering problem. Any solution (such as MOSE) that involves changing how water is exchanged between the lagoon and the Adriatic will necessarily also impact the lagoon's ecology and geometry, key elements that help to control the way storm surges propagate to the city.

The book does a good job of explaining why Venice floods, what remedies have been applied in the past, and which may be important in the future. It describes why the solution to the problem of flooding has to be more holistic than simply blocking storm surges from entering the lagoon. It is also stunningly illustrated, with numerous drawings and photographs that clearly illustrate the concepts discussed as well as the city's renowned beauty. Despite its title, *The Science of Saving Venice* is not at all scientific: beyond a few figures, a glossary, and a list of some relevant Web sites and organizations, it offers no technical information, no quantitative analysis, nor any references to published, peer-reviewed work. Readers interested in the science and engineering involved will have to track that down independently.

A frequent sight. St. Mark's Square is now inundated more than 60 times a year.

Such shortcomings do not, however, negate the book's principal values. The authors provide the lay reader with a broadly useful foundation for understanding the complexity of the situation Venice now faces and explain why saving the city for future generations depends largely on how well we achieve a good scientific understanding of its tremendously complex natural environment. In the spirit of the best field guides for travelers, the book offers an informative and engaging account of an issue related to one of the world's most precious cities—an account that can only help increase public awareness of Venice's plight and thereby contribute to its rescue.

–JESSE SMITH

10.1126/science.1116750

SCIENCE AND GOVERNMENT

In Search of the Best Grant System

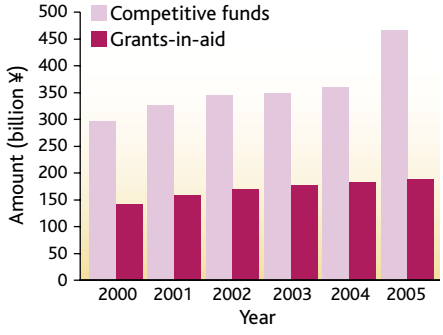
Tasuku Honjo

The Japan Society for the Promotion of Science is known internationally for providing fellowships (~6570 per year) for doctoral students and postdoctoral researchers. Less known is that JSPS is Japan's largest research funding agency, distributing ~¥128 billion (US$1.2 billion) for competitively reviewed grants annually, funded by the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) (*1*). JSPS also promotes and supports international collaborations in the form of travel grants and funding for bilateral exchange programs.

From 1995 (when the Science and Technology Basic Law was passed) until now, the budget for MEXT's competitive research grants has doubled (see figure, right) (*2*). MEXT provides the majority of research grants to the Japanese academic community. They cover the natural sciences, engineering, social sciences, and humanities. In 1998, MEXT decided to convert JSPS into a funding agency and transferred a major portion of its research grant administration to it.

Establishing a fair grant-review system is essential for any funding agency. Nevertheless, it is impossible to satisfy everybody. In the past, JSPS's grant reviewers were automatically picked from a list of candidates recommended by academic societies. This was not well accepted by the scientific community, because neither names nor selection methods of some of the societies were disclosed. Another concern was that the process was in the hands of administrators who were not scientists.

In 2003, JSPS established the Research Center for Science Systems to provide advice from a scientist's viewpoint on all facets of JSPS's program, especially the grant review process, e.g., preparing evaluation criteria, listing reviewer candidates, and recommending new grant formats and systems. The center's staff consists of three program directors and 96 program officers, who supervise the entire review system and choose reviewers for JSPS grant applications (*1*). The program officers are highly qualified scientists recommended by universities and research institutes. They represent eight research domains: the humanities; social sciences; mathematics and physics; chemistry; engineering; agriculture; biology; and medical sciences, including dentistry and pharmacology. Every other week, the program directors, 16 senior program officers (two for each domain), and members of the administrative staff discuss the center's program and current issues. The program officers in each of the domains hold their own meetings once or twice a month. They serve for 3 years, coming into the center 1 or 2 days a week to consult.



Japanese research funding. Total competitive research funds from the Japanese government and grants-in-aid from MEXT.

Nothing is more important than having the best scientists serve on the review committee. The center has built a database of 17,000 scientists with established track records in winning grants and publishing papers. In this fiscal year, 2000 new reviewers were chosen from this database. Our current system has two layers. First is a document review of mailed-in applications, with six reviewers assigned to each application. Next is a panel review conducted by 12 to 22 senior scientists who make the final decisions. The center's program officers monitor outcomes of the document and panel reviews by attending all review-panel meetings. Feedback reports from them are compiled and used to improve the evaluation process and the quality of reviewer performance. Applicants are given their rated scores and comments. However, providing a detailed report to each applicant would not be an easy task, especially given the fact that JSPS handles some 80,000 applications within a 4-month period each year. In addition, care must be taken to avoid placing an excessive burden on reviewers so as not to discourage them from contributing their precious time.

The center is also expected to serve as a think tank for the entire grant system supported by the Japanese government. Since radical reform of the university system started in 2004, there has been an increasing demand for external funding. Although universities are acquiring more resources from industry, MEXT's grants-in-aid are still the primary source for funding academic activities, especially bottom-up proposals from scientists in all disciplines. As basic government funding is gradually shrinking, universities are seeking grants for investigator-initiated proposals. A serious concern among university scientists is finding a balance between funding for such proposals and for mandated research. Recent pressure by industry has altered this balance in favor of top-down projects in such fields as bioscience, information technology, nanotechnology, and environmental science. I propose to even this balance while doubling the grant-in-aid budget over 10 years.

During the past decade, technological advances have not only heated competition but also have raised laboratory operating costs. Many top-ranked laboratories require at least a few grants to sustain their programs. However, current policy strictly prohibits multiple awards to one principal investigator. It will be essential to increase the number and amount of grants to cover the full scope of the awardee's activities. It will also be important to enhance the categories dedicated to young researchers and to establish a new category for encouraging female investigators. In this context, there is a critical need to continuously evaluate the function and performance of each grant category along with the appropriateness of resource allocations.

Another concern is overlap of funding for top-down projects from multiple government agencies. In response, we will draft and propose an action plan for tackling these issues by March 2007. We are searching for the optimum grant system, although a solution that satisfies most people may still be many years in the offing.

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10.1126/science.1115377

PHYSICS

A Quantum Critical Route to Field-Induced Superconductivity

A. P. Mackenzie and S. A. Grigera

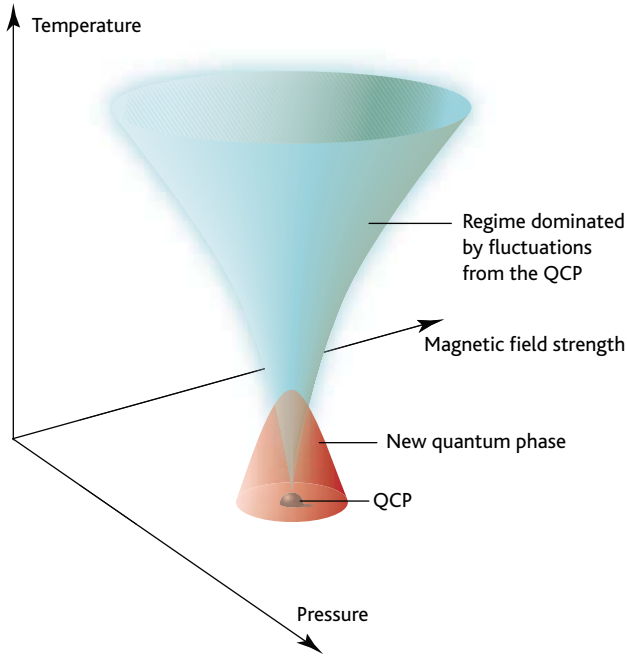
These are exciting times for superconductivity research and for studying the physics of materials with strongly interacting electrons. We are witnessing a revolution in our understanding of new and exotic forms of superconductivity, and novel behavior is being discovered every few months. The report by Lévy *et al.* on page 1343 of this issue (*1*) describes a particularly important development in a line of investigation focused on the concept of quantum criticality. At a typical thermal phase transition, such as the ferromagnetic transition of iron, thermal energy produces strong fluctuations that drive the phase change from ferromagnetism (when the material is spontaneously magnetized) to paramagnetism (when the individual magnetic dipoles only align in response to an external field). Over the past decade, we have realized that it is possible to use some external parameter such as high pressure to tune the critical temperature of a phase transition toward absolute zero, producing a quantum critical point (QCP). The idea of a fluctuation-driven transition remains, but the fluctuations become dominantly quantum mechanical rather than thermal. This has profound consequences for our theoretical understanding and has generated a mini-industry within the physics community (*2*).

Enhanced online at www.sciencemag.org/cgi/content/full/309/5739/1330

The existence of a QCP also has striking effects on the observable physical properties of the system. It gives rise to previously unexpected behavior in the quantum critical region (see the figure), which, paradoxically, might even extend to room temperature and beyond. The consequences for the low-temperature properties can be more spectacular still. In 1998 the Cambridge group studied the formation of QCPs by tuning the antiferromagnetic transition in a series of specially chosen metals. Before the critical point was reached, the systems underwent a second phase change to a superconducting state (*3*). This was a real revelation, because the discovery of new superconductors has traditionally been an extremely subtle business. Although there was some guidance from empirical rules of thumb, notably those based on understanding of crystal structure, actual discoveries were more or less serendipitous. Now we had a new set of guidelines: Make QCPs and search for novel superconductivity in their vicinity.

The quantum critical approach has led to a number of important discoveries. Advances have been made concerning our understanding of the heavy fermion superconductor $CeCu_2Si_2$ (*4*), and new materials have been discovered in which superconductivity coexists with ferromagnetism (*5*). In each of these cases, QCPs were produced by the application of high hydrostatic pressure. A related question that several groups began to address was what would happen in special circumstances in which a magnetic field was used to tune the criticality. Would superconductivity or something else be the result? Superconductivity would certainly be disfavored in these circumstances, because superconductors screen out magnetic fields, and this costs energy. Indeed, the first evidence for phase formation in the vicinity of magnetically tuned QCPs involved novel nonsuperconducting behavior (*6*, *7*). Nothing in this work proved that field-induced superconductivity is impossible, but it seemed like quite a long shot, because even if there is a net energy gain from forming a superconducting state, it must compete with the net energy gain from forming other phases.

This, then, is the context in which Lévy *et al.*'s work is important. Soon after the discovery of pressure-induced superconductivity in UGe_2 (*5*), Aoki *et al.* discovered superconductivity at ambient pressure in the related material URhGe (*8*). When superconductivity was first discovered in UGe_2, it was assumed to be related to the ferromagnetic transition going quantum critical. Later, however, evidence began to emerge that the driving transition might be one between two subtly different forms of ferromagnetic order. Working with URhGe, Lévy *et al.* noticed signs of a magnetic transition at high fields and decided to investigate further (*1*). Using neutron scattering, they established that it involved a rotation of spin direction within the ferromagnet, and that this might be the source of a metamagnetic quantum critical endpoint (*9*). Low-temperature measurements on their best crystals then revealed that although the original superconductivity is suppressed by a magnetic field in the usual way and



Quantum criticality and phase formation. A schematic quantum critical phase diagram. The quantum critical point (QCP) is formed by tuning some combination of external parameters such as pressure and magnetic field. The presence of strong quantum fluctuations and the depressed energy scales increase the susceptibility of the system to entering a state with a class of order that did not form part of the original phase diagram (red cone). Empirically, the new phase is found surrounding the QCP, which is therefore not actually reached.

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disappears at 2 T, even stronger superconductivity reappears at the astonishingly large field of ~10 T.

Field-induced superconductivity had been seen before but could be explained in a rather conventional way, with the external field simply canceling the internal field that exists in a ferromagnet. Thanks to their neutron-scattering work, Lévy *et al.* can give convincing arguments for an entirely different, quantum critical origin for what they are seeing. A common feature of novel quantum order near QCPs is that the new states are fragile and are rapidly destroyed if the crystals are imperfect and contain disorder. This is also the case here, so in addition to performing difficult high-precision physical measurements, Lévy *et al.* had to use state-of-the-art crystal growth to obtain material of the requisite purity. Their work is an experimental tour de force.

In a more general sense, the importance of these advances is the construction—arguably for the first time in this field—of a framework for discovery. Careful work producing and then investigating QCPs is leading to a series of experimental breakthroughs. This does not, however, mean that we now understand everything. For example, it is widely assumed that quantum critical superconductivity is driven by magnetic fluctuations. Although this is very likely to be true, further work will be needed before the statement can be made with absolute certainty. Coupling between magnetic and structural properties ensures that phonons will also still play some role, and the strength of this coupling has not yet been determined in most cases.

A broader question is whether one needs a fluctuation-driven description at all. QCPs are indisputably accompanied by strong quantum fluctuations, but the mean-field free energy landscape also becomes very flat in their vicinity, increasing the susceptibility to phase changes in which the role of fluctuations is much less clear. The fact that these new phases need not always be superconducting gives promise for advances that will continue to surprise. Progress is rapid and exciting, but there is still much to be achieved.

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10.1126/science.1117436

MICROBIOLOGY

Exploring Microbial Diversity—A Vast Below

T. P. Curtis and W. T. Sloan

Exploring microbial diversity is becoming more like exploring outer space with soil representing a "final frontier" that harbors a largely unknown microbial universe. There are more than 10^{16} prokaryotes in a ton of soil compared to a mere 10^{11} stars in our galaxy. Astronomers have wisely inferred the population of celestial objects by mathematical inference. Now microbiologists are following suit, adopting a similar strategy to estimate the number of prokaryote taxa in soil. As shown by Gans *et al.* on page 1387 (*1*), the inferred diversity is staggering—higher than previously thought by almost three orders of magnitude.

The extent of prokaryote diversity has been hotly debated and rightly so. Microbial communities are central to health, sustainable cities, agriculture, and most of the planet's geochemical cycles. Prokaryote communities are also reservoirs for the discovery of new drugs and metabolic processes. As with any reservoir, its size is important.

Measuring the reservoir of prokaryotic diversity is not a trivial task. There is broad agreement that the key is to eschew the organisms themselves and to focus instead on their DNA. If DNA from a single organism is purified and heated, the strands of the double helix separate or "melt." If you then slowly cool the DNA, the strands will reassociate or reanneal, and the rate at which this happens is affected by the size and complexity of the DNA. Big and complex DNA reanneals slowly. This fact has been used for the past four decades to estimate the size and complexity of genomes from individual organisms. Around 15 years ago, Torsvik *et al.* (*2*) reasoned that pooled genomic DNA from a microbial community might reanneal like the DNA from a large genome. Indeed, they showed that DNA extracted from soil reassociated slowly—so slowly that it resembled a genome that was 7000 times as large as the genome of a single bacterium. It follows that there could have been at least 7000 different prokaryote taxa in the sample of soil that they analyzed. At the time, this was considered a mind-boggling number. Even ecologist E.O. Wilson speculated that "microbial diversity was beyond practical calculation" (*3*).

There is, however, another way to estimate prokaryotic diversity in the environment. A biological community has a characteristic abundance distribution of its member species. The observation and contemplation of these distributions have a rich literature in conventional ecology that is helping rescue microbial ecology from the conundrum of how to estimate diversity. In principle, if you know the shape of the taxa abundance distribution curve, you know the diversity. But there is a catch: Typically, for large organisms, species abundance distributions have been determined by assessing the abundance of almost all of the species in a sample, which means that you must already know the number of species. In the absence of such information, one still can draw upon certain theoretical considerations (*4*), assume that a particular species distribution pertains, and then make an estimate (*5*). Alternatively, you can fit a curve to the data you have to make an estimate (*6, 7*). The latter approach has great merit, but gathering enough data to make a sensible decision about the underlying species distribution pattern is problematic. At present, most microbiologists attempt to estimate diversity by looking at a gene that occurs in all cellular life forms. They infer diversity from the number of different variants that can be cloned from a sample of environmental DNA. Unfortunately, the number of clones analyzed is typically small (tens to hundreds) compared to the number of individual microbes being analyzed (billions or trillions). This is like randomly sampling a bus load of people and then trying to infer the diversity of all people in the world. You would not expect to find many Lithuanians.

Gans *et al.* (*1*) and others (*8*) realized that the pattern of DNA reassociation

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kinetics reflects the underlying distribution of similar sequences, and hence likely reflects genomic diversity. However, the authors have gone further and show that there is probably enough data in published DNA reassociation curves for bacterial communities to allow discrimination between different possible species abundance curves. By applying new mathematical treatment of data, the authors generate abundance curves, the most plausible of which suggests that there could be 10^7 distinct prokaryote taxa in 10 grams of pristine (free of chemical contaminants) soil (see the figure). Moreover, rare organisms comprise most of this diversity. They further determine that most of these rare organisms could be wiped out by heavy metal pollution of the soil.

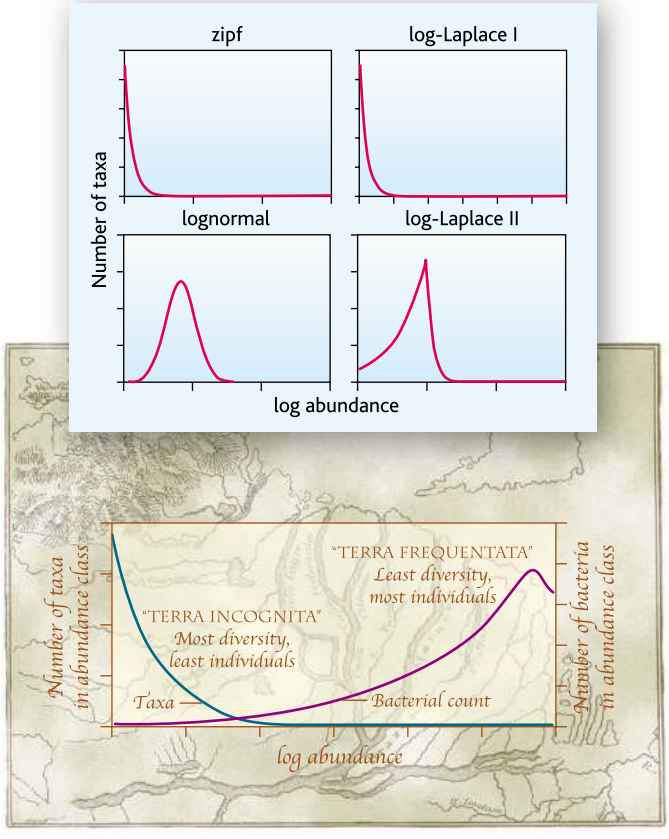
The jargon and mathematical notation of taxa abundance distributions can be obscure. However, when presented graphically the curves are as simple and as useful as an outline of an unexplored continent (see the figure). Thus, power law distributions (like the zipf distribution) simply describe exponentially increasing numbers of species at exponentially decreasing abundances. On the other hand, lognormal distributions suggest that at lower abundances, the number of rare species start to decrease. It is no mystery therefore that wiping out rare species, as in the case with heavy metal soil pollution, diminishes the ability to distinguish between the two different situations. Gans *et al.* (*1*) point out that the log-Laplace distribution (see the figure) is theoretically attractive because it derives from an ensemble of lognormal distributions but, as such, it can be made to look a little bit like either a lognormal or a zipf and therefore (unsurprisingly) fits well in all circumstances.

The work of Gans *et al.* (*1*) simply represents a rough "map" of the whole microbial community of a sample of soil, which may be far more useful right now than an exquisite description of just part of it. One could argue that the estimates might be affected by reassociation of sequences common to many taxa. And the curves presented by the authors certainly constitute tremendous extrapolations (though they are supported by simulations). However, such quibbles are immaterial if the overall picture is even approximately correct. An imperfect, simple map of an entire region can guide an explorer with more certainty than a perfect representation of one creek. The explorer, thus guided, will produce better maps that will better guide more explorers, and so on. Dispensing with the map is like "wildcatting" or "swashbuckling" for diversity: exciting and profitable if you make a strike, but ultimately subject to diminishing returns and the inevitable disinterest of your sponsors. (Sir Walter Raleigh, a 16th-century English swashbuckler, failed to find El Dorado on the Orinoco and was later beheaded. Today's sponsors are a little more understanding.) Rational plans and costs for exploring the microbial frontier require a proper mathematical framework for this task. Indeed, we cannot even resolve some very basic questions.

Thus, exponentially increasing numbers of taxa at exponentially decreasing abundances means that, in random samples of diversity, a few abundant taxa can turn up again and again. Resolving whether the recurrence of abundant taxa is an artifact of sampling (the equivalent of the mapless explorer going around in circles) or a genuine reflection of low diversity (*9*) is important in resolving the debate on the extent of prokaryotic diversity.

The Gans *et al.* (*1*) report is part of a wider shift in the study of microbial communities. At present, the science is primarily observational. This study, together with other work (*4–7, 10*), shows that our powers of observation can be vastly enhanced by sensible mathematical techniques. But observation per se will never be enough to explain the microbial world, any more than we can explain the universe by just looking at the stars. A recent flurry of papers on taxa-area relationships (*11–14*) may point to a way forward. These papers are also observational and cannot explain the microbial world either. However, the emergence of common patterns in data from a number of environments hints at deeper and perhaps universal, underlying processes that could be expressed formally as explicit mathematically supported theory.

In the world of microbial ecology, we need theory very badly. Almost any consequential microbial community will have 10^{10} to 10^{17} bacteria that could compose more than 10^7 differing taxonomic groups and countless functional groups. It seems remarkable that we should even contemplate trying to understand such vast systems without recourse to some form of theory. At the very least we may hope to not only "substitute one theory for many facts" (*15*) (and we are accruing facts at an astounding rate), but also quantitatively guide our exploration, facilitate the application of the methods we have, and develop new tools. We might ultimately hope to develop greater powers of prediction. This would be a revolutionary development, but not necessarily an easy one. The findings of Gans *et al.* place a special duty on theorists in this area of ecology. The challenge is to offer mathematical guidance in a world we can barely perceive and not to explore ultimate causes for patterns we can readily observe. We therefore need to develop simple (*16*) models that we can calibrate and then improve. Like the "map" outlined by Gans *et al.*, simple theories will mark the beginning, not the end, of the rational exploration of this fron-



Mapping microbial diversity. The relative abundance of microbial taxa can be described with abundance distributions. The total number of species is the area under the taxa plot line. The precise shape of any given curve will depend on the parameters selected. In all cases, the majority of the biomass that we can most readily observe (Terra Frequentata) make up a minority of the diversity. Most taxa are very hard to find by random sampling (Terra Incognita).

tier. Exploring rationally might lead us to a healthier, cleaner, and more productive world and ensure that we don't lose our heads or our minds, walking in circles on the edge of a final frontier looking for an "El Dorado."

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CHEMISTRY

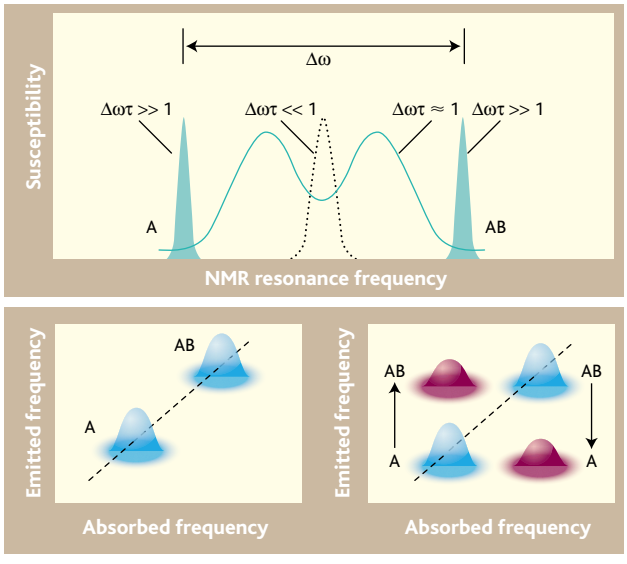
Ultrafast Chemical Exchange Seen with 2D Vibrational Echoes

Dana D. Dlott

Fast exchange among chemical species is ubiquitous in chemistry, as typified by the reaction scheme taught in freshman chemistry: A + B ↔ AB → products, where A and B rapidly fluctuate between individual species and AB complexes. If A and B are dilute they meet infrequently, but if B is the solvent then exchange can occur within picoseconds. It is straightforward to measure equilibrium concentrations, but tricky to measure complexes flickering in and out of existence. On page 1338 of this issue, Zheng *et al.* report an important advance in capturing these kinds of rapid chemical exchange reactions with ultrashort infrared laser pulses (*1*). In a remarkable example of simultaneous breakthroughs, Hochstrasser's group at the University of Pennsylvania also reports similar measurements in this week's *Proceedings of the National Academy of Sciences of the USA* (*2*).

The breakthroughs (*3*) in the 1920s that allowed solvent exchange reactions to be studied involved millisecond mixing of reagents. Microsecond relaxation methods were developed in the 1950s (*3*), where the formation of chemical complexes was perturbed by a temperature jump or with flash photolysis. Today femtosecond flash photolysis is frequently used. However, these are perturbative methods that do not measure chemical exchange in equilibrium.

In 1953, Gutowsky, McCall, and Slichter (*4*) developed the first NMR (nuclear magnetic resonance) spectrometer capable of resolving multiplet splittings in liquids. Today we call this 1D-NMR (one-dimensional NMR), and this work put NMR on the map, because it showed how to deduce molecular structure from spectra of ^{1}H, ^{13}C, ^{17}F, ^{31}P, and other atoms with magnetic dipoles. Gutowsky and Saika then showed how NMR spectra measure chemical exchange (*5*). As seen in the top part of the figure, when species A and AB with NMR transitions split by a frequency difference Δω undergo chemical exchange, the peaks shift toward each other. This effect is evident when $\Delta\omega\tau \approx 1$, where τ is the time constant for exchange; otherwise, exchange is too fast or too slow to measure. A typical value of Δω is 500 Hz, so chemical exchange faster than milliseconds is not directly accessible by 1D-NMR. It is also difficult to measure exchange at a single temperature with 1D-NMR, so usually temperature is varied to obtain spectra from the slow to the fast limits.



Mapping a chemical swap. (**Top**) In 1D-NMR, A and AB peaks are split by Δω if the time for chemical exchange τ is long ($\Delta\omega\tau >> 1$). If $\Delta\tau \approx 1$ the peaks broaden and move toward each other, coalescing into a single average peak when exchange is fast ($\Delta\omega\tau << 1$). (**Bottom**) In 2D spectroscopy, formation of complexes is indicated by off-diagonal peaks that grow as time passes following the initial vibrational excitation.

A breakthrough was made in 1979 by Meier and Ernst (*6*) with 2D-NMR. In 2D-NMR, a radiofrequency pulse is used to excite nuclear spin transitions. After some delayed pulses, a spin-echo pulse is emitted. It is called an "echo" because the intermediate pulses reassemble the dephasing spin excitation into a copy of the original pulse. As shown in the bottom part of the figure, in a 2D spectrum one axis is the absorbed frequency and the other the emitted echo frequency. When A or AB absorbs and then emits an echo, peaks are observed on the diagonal, but if A absorbs and subsequently becomes AB, then a new peak grows in off the diagonal. In fact, one off-diagonal peak measures A + B → AB and the other AB → A + B, all at a single temperature. The time resolution of the 2D technique is limited to 100 nanoseconds by the radiofrequency pulse durations needed to manipulate the spins and generate the echoes.

Zheng *et al.* (*1*) have demonstrated 2D measurements of chemical exchange between phenol and benzene solvent by means of femtosecond infrared (IR) vibrational echoes. Vibrational echoes are analogous to spin echoes but there are significant differences that make the experiment challenging. The Gutowsky NMR experiments were enabled by advances in magnet homogeneity and electronic amplifiers. The Zheng *et al.* vibrational echo experiments are enabled by state-of-the-art femtosecond IR laser technology, nanopositioners, and IR detector arrays. Vibrational echoes do not require a magnet because molecules have intrinsic vibrational splittings. Spin levels are evenly spaced but vibrations are anharmonic, so vibrational splittings become smaller as energy increases. Spin echoes are emitted nondirectionally, but because IR wavelengths are smaller than the sample,

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vibrational echoes are emitted in specific phase-matched directions. The IR pulses were four cycles in duration, or about 50 femtoseconds; the equivalent NMR pulse would be 10 nanoseconds. NMR linewidths result mainly from dipolar broadening and molecular rotations, but IR linewidths result from energy transfer among vibrations, molecular collisions, and variations in hydrogen bonding.

Vibrational echoes were observed first in the gas phase by Brewer and Shoemaker in 1971, using microsecond-duration IR pulses (*7*). It was not until the 1990s that IR pulse generation technology made condensed-matter measurements possible. The Fayer group (*8*) studied inorganic complexes and biomolecules in solution using IR pulses from a free-electron laser and tabletop lasers. Multidimensional IR with heterodyne detection was subsequently introduced by the Hochstrasser group (*9*).

In the technique used by Zheng *et al*., an IR pulse excites the OH-stretch of phenol. If phenol forms a complex with benzene solvent, the vibrational echo creates an off-diagonal peak. The useful time range is about 20 femtoseconds to 100 picoseconds. The spin excitations of NMR do not affect most chemical reaction rates, but many reactions are perturbed by vibrations. The vibrational echo experiments simultaneously record off-diagonal peaks in both vibrational ground states and excited states. In the phenol-benzene system studied by Zheng *et al*., the rates of chemical exchange appear unaffected by the OH-stretch excitation.

The vibrational echo technique of Zheng *et al*. (*1*) and Kim and Hochstraser (*2*) is a general method for measuring ultrafast chemical exchange of mocules in solution. From the viewpoint of chemical reaction dynamics, it is remarkable that the exchange rate can be measured with or without vibrational excitations. The phenol-benzene studies of Zheng *et al*. and the methanol-acetonitrile studies of Kim and Hochstrasser both involve the making and breaking of hydrogen bonds in organic solvents. If the technique can be extended to measure exchange between aqueous solvents and biomolecules, the potential impact will be unlimited.

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CELL BIOLOGY

A Gradient Signal Orchestrates the Mitotic Spindle

Paul R. Clarke

Eukaryotic cell division is entrancing when observed through a microscope. In a dramatic prelude, the internal structure of a cell is reorganized and pairs of duplicated chromosomes become arranged in the middle of the cell. Then each pair is separated into chromatids that are segregated one to each side, so that when the cell divides, each "daughter" cell receives an identical set of genes. This formidable feat is achieved by the mitotic spindle, a precision machine made from a bipolar array of microtubules that are focused at each end of the spindle by a centrosome or spindle pole body. Microtubules are themselves dynamic polymers that interact with chromosomes in the middle of the spindle and provide tracks to separate them toward the poles. Without the spindle, cell division would be impossible, and subtle defects in its function are likely to be involved with the genomic instability associated with cancer.

The mechanisms that orchestrate assembly of the mitotic spindle have been somewhat of an enigma. It has been proposed that signals emanating from chromosomes that promote microtubule growth play a key role (*1*). On page 1373 of this issue, Caudron *et al*. (*2*) provide the clearest evidence yet that spindle assembly is coordinated by the generation, at chromosomes, of an intracellular gradient of the active guanosine triphosphate (GTP)–bound form of Ran, a small GTPase of the Ras superfamily present in all eukaryotic cells.

The concept of signaling gradients is a familiar one in animal development (*3*). Release of a diffusible and slowly degraded chemical, or morphogen, from a specific site can produce an extracellular concentration gradient that provides positional information to cells. The effect on a particular cell (for example, inducing differentiation) is determined by the cell's threshold in the response to the graded signal. If there are multiple thresholds, then the gradient can produce patterns of different cell responses. These may be limited to precise concentrations of the morphogen, and hence a precise position within a developing tissue. Intracellular gradients that provide positional cues can be generated through subcellular localization of mRNA, such as the localization of *bicoid* mRNA at the anterior pole of the *Drosophila* oocyte. Local translation subsequently produces a gradient of bicoid morphogen during early development (*4*).

Intracellular gradients can also be generated by enzyme activity. In this case, spatial information is provided by the concentration gradient of a diffusible substrate generated by a fixed enzyme. For instance, if phosphorylation of a diffusible protein is catalyzed by a localized protein kinase, and if the opposing protein phosphatase is dispersed, then a gradient in the phosphorylation status (and therefore in the activity of the substrate protein) can be generated (*5*). Such a reaction-diffusion process creates a steadily changing gradient that is distinct from sharp concentration differences due to compartmentalization, such as the difference in ion concentration across an impermeable membrane. Previous work has shown, by mathematical simulation combined with molecular experiments using fluorescence reporters, that such a chemical gradient can be generated by phosphorylation of the microtubule-binding protein stathmin (*6*).

Now, Caudron *et al.* (*2*) have used a similar dual approach to examine the gradient of Ran-GTP generated by mitotic chromosomes in *Xenopus* egg extracts (a system commonly used to study mitosis). Ran-GTP releases factors that are required for spindle assembly from inhibitory complexes that also contain proteins involved in nuclear transport called importins or karyopherins. The gradient of Ran-GTP directed away from chromosomes is proposed to provide a positional signal that causes changes in microtubule dynamics and organizes the spindle around chromosomes. The key assumptions of this model, based on good

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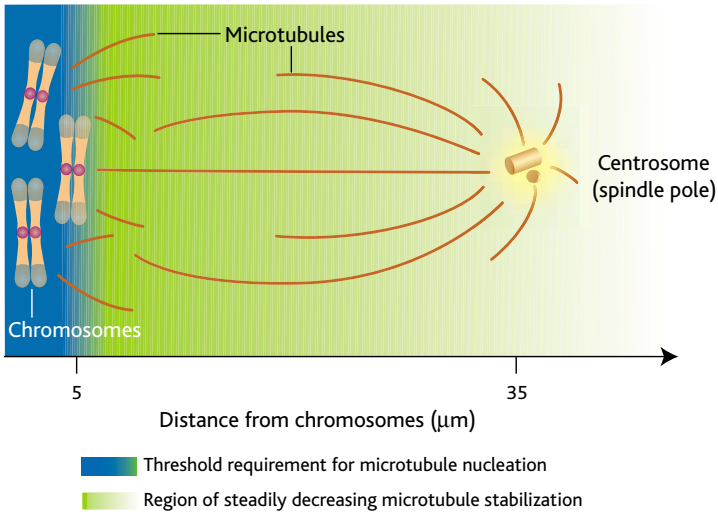
experimental evidence, are that RCC1 (the nucleotide exchange factor that generates Ran-GTP) is localized predominantly to chromosomes, while the hydrolysis of diffusible Ran-GTP is catalyzed by a largely dispersed GTPase-activating protein, RanGAP1, thereby forming a reaction-diffusion system that can be described mathematically.

The use of mathematical simulation to describe a process is sometimes viewed with skepticism by molecular and cellular biologists who regard such models as drastically oversimplified in terms of the molecules involved and their functions, and lacking in predictive power that would help design further experiments to test the hypothesis. Nonetheless, even skeptics can appreciate the use of mathematics to provide a clear framework on which to organize ideas and formulate general principles. Building on previous simulations (*7*) and experimental evidence (*8*), Caudron *et al.* confirm that a steep gradient of free Ran-GTP is generated around chromosomes in *Xenopus* egg extracts. In addition, they demonstrate the generation of a longer range gradient of Ran-GTP that is in a complex with importin-β. It is this gradient that the authors suggest is more informative about the concentration of released spindle assembly factors.



Chromosomes generate a chemical gradient that organizes the mitotic spindle during cell division. Microtubule nucleation occurs only within a region close to the chromosomes, as a result of an ultrasensitive response to a gradient of Ran-GTP–importin-β. Microtubule stabilization works at greater distances from the chromosomes and responds linearly to the gradient. These responses may reflect differing sensitivities of effectors in each of the processes to Ran-GTP, so that spatial information is provided by the gradient. Half of the assembling spindle is represented.

Caudron *et al.* show that microtubule nucleation is highly dependent on a threshold level of Ran-GTP–importin-β being exceeded and that this only occurs in the vicinity of chromosomes. On the other hand, stabilization of the dynamic "plus" (growing) ends of microtubules responds proportionately to Ran-GTP–importin-β concentration and is extended over longer distances, as far as the centrosomes (see the figure). Varying the Ran-GTP–importin-β gradient experimentally by adding regulators of Ran-GTP production or stability disrupts proper bipolar spindle assembly around chromosomes. The inference is not only that the generation of Ran-GTP is required, but that the correct gradient of concentrations is important to provide the proper spatial cues for different reactions during the assembly of the spindle.

One clear prediction of this signaling gradient model is that factors involved in microtubule nucleation or plus-end stabilization will differ in their interaction with importin-β (or that they are complexed to different members of the importin/karyopherin family) and are released at different threshold concentrations of Ran-GTP. This is analogous to thresholds in the response of cells to morphogens. Because microtubule interactions with chromosomes are disrupted by altering the gradient, Caudron *et al.* propose that the stabilization of microtubules at some distance from chromosomes promotes their growth toward the chromosomes, and this promotes their "capture" by microtubules.

In an alternative "search-and-capture" model for spindle assembly, stochastic microtubule growth from centrosomes would be stabilized by interactions with specific protein complexes, particularly at kinetochores [structures on chromosomes that are important for chromosome movement toward the spindle poles and to which microtubules are attached (*9*)]. In contrast to the signaling gradient model, search-and-capture does not require longer range effects of chromosomes on microtubule growth. However, chromatin lacking kinetochore regions can organize spindle assembly in the absence of centrosomes in *Xenopus* egg extracts (*10*), and theoretical modeling indicates that the search-and-capture process alone would be too slow (*11*). Even so, there is recent evidence that, in addition to localization of RCC1 across mitotic chromosomes, a subset of RanGAP1 molecules together with a Ran binding protein called RanBP2 are localized to kinetochores through the interaction of Ran-GTP with the karyopherin Crm1 (*12*). These localized molecules are necessarily not considered in the simplified model used by Caudron *et al.*, yet they appear to have an important function in kinetochore attachment of microtubules. Indeed, it is likely that Ran has a fundamental role in the interactions of microtubules and chromosomes that is conserved in eukaryotes (*13*).

The signaling gradient model of Caudron *et al.* may therefore not be the complete picture. Both the effects of a widespread Ran-GTP gradient and specific localized functions of Ran-GTP could be incorporated in a biased search-and-capture mechanism (*11*) in which the Ran-GTP gradient permits release of factors that stabilize or nucleate microtubules while the interactions of microtubules with kinetochores are controlled by localized complexes dependent on Ran-GTP. And although *Xenopus* egg extracts are a very valuable model for mitotic spindle assembly, it remains to be seen if the gradient mechanism is necessary in cells where there are other spatial constraints on spindle assembly and orientation. Nonetheless, the work of Caudron *et al.* provides the clearest portrait yet of how Ran orchestrates the overall process of mitotic spindle assembly, and it illustrates very nicely how an intracellular chemical gradient can provide spatial information. It will be interesting to see whether this is a general principle for the organization of structures within cells.

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edited by Edward W. Lempinen

SCIENCE AND DIPLOMACY

AAAS S&T Policy Fellows Risk Their Lives to Rebuild Iraq

Alex Dehgan remembers the sound—it was like a car door closing. And then a few seconds of waiting, helplessly, never knowing whether the mortar would explode nearby, or at a distance. Some nights he went to sleep at his quarters in the fortified Green Zone wearing his flak jacket like a blanket.

It was not what he envisioned when he first applied for the AAAS Science & Technology Policy Fellowships. But like a small corps of current and former Fellows, Dehgan, as a Diplomacy Fellow at the State Department, made a remarkable commitment to rebuilding war-torn Iraq.

AAAS Diplomacy Fellow Krista Donaldson, also at the State Department, made two extended trips to Iraq, using her expertise as a mechanical engineer to help bolster the nation's electrical grid. Peter Smallwood, a 2003–04 Congressional Fellow, spent nearly 10 months in Iraq managing the U.S. State Department's program to direct former Iraqi weapons experts and other scientists into reconstruction efforts. Dehgan, after 5 months in Iraq last year, worked with a half-dozen AAAS S&T Fellows from the Departments of State and Defense to organize a virtual library of science and engineering publications that will soon serve hundreds of Iraqi scholars and students.

Dehgan directed the weapons scientist program in its first months of operation—an effort seen as helpful in stabilizing the nation and keeping the scientists from going to work for hostile forces.

Despite the extreme risks, Dehgan said recently, "I don't regret it, so long as others can continue working on building science in Iraq, and so long as the Iraqi people have hope in their future. But it was hard facing these fears all the time without the support of my family—I could not tell them of the dangers that I was in, or that I was even in Iraq—because I didn't want them to worry."

Mechanical engineer Krista Donaldson on duty in Iraq last winter.

After winning the fellowship, Dehgan was assigned to the State Department's Bureau of Near Eastern Affairs and volunteered to work with the Coalition Provisional Authority, which governed Iraq after Saddam Hussein's fall. Though he was based in the Green Zone, he frequently ventured outside the walls to work with Iraqi colleagues at the program's headquarters.

In a recent interview, he described narrowly avoiding injury in nearby explosions, getting caught in cross-fire, and living with death threats. "Even upon leaving, my plane and the airport came under fire," he said. "The stress never stopped until I was in the United Kingdom, where I was briefing the British Ministry of Defense—and even then, it took months before I could relax."

Peter Smallwood (left), a 2003–04 Congressional Fellow, and Alex Dehgan, a 2003–05 Diplomacy Fellow, at a Baghdad restaurant in May 2004.

Smallwood, a behavioral ecologist, served during his fellowship as an aide to U.S. Senator Joe Lieberman (D-Conn.) on environmental issues. He and Dehgan were friends, and when the fellowship ended, he signed on with the State Department's Iraq Reconstruction Management Office. Dehgan came home in mid-June 2004; Smallwood was sent to replace him in September.

By the time he reported for work in Baghdad, the security situation had deteriorated further, and leaving the Green Zone was still more dangerous. "Everyone deals with stuff in different ways," Smallwood said. "I adapted to my own security situation pretty well. Much more difficult for me was making decisions that affected the security and safety of others. That's not something I have any training for."

During his time in the war zone, he too dodged bombs and survived attacks on the Green Zone—but two friends and a half-dozen acquaintances were killed. He returned to the United States at the end of June and will resume teaching in the fall semester at the University of Richmond.

After his return home, Dehgan continued to work with scientists in Iraq and the Middle East. He also was an organizer of the Iraq Virtual Science Library, which is slated to go online this fall to help replace the libraries and academic institutions that were looted in Iraq. Other AAAS Fellows who have collaborated on the program: Susan Cumberledge, D.J. Patil, and Ben Perman at the Defense Threat Reduction Agency; Ranjiv Khush in the Office of the Science and Technology Adviser at the State Department; Kwabena Boakye-Yiadon at the Office of the Secretary of Defense; and Barrett Ripin, a 2001–02 Diplomacy Fellow and now a senior science diplomacy officer at State.

The U.S. National Academy of Sciences, Sun Microsystems, and an increasing number of scientific journal publishers also are partners in the project.

With the yearlong fellowship ending this month, most of the Fellows are returning to labs, teaching, and other jobs. Dehgan has not decided what he'll do next. He received a letter of commendation from the Department of Defense, and in July he received the State Department's Superior Honor Award, given "in recognition of a special act or service or sustained extraordinary performance."

The same month, he finally told his parents of the work he'd done in Iraq.

EDUCATION

AAAS/Packard Graduate Scholars Blaze Ph.D. Trail

Virtually everyone who has gone to graduate school in science, engineering, or math knows that the road to an advanced degree is rigorous, requiring long hours over several years and the ability to stay focused even in periods of struggle. But scholars coming from historically black undergraduate schools to major research institutions often face additional challenges: isolation, a lack of role models, and, sometimes, disrespect.

The challenges were at the forefront of discussion when more than 50 young researchers in the elite AAAS/Packard Graduate Scholars Program convened last month in Monterey, California, for their annual meeting. They are ambitious scientists and researchers, many already on a track to leadership, and during 3 days of meetings and panels they did what grad students usually do when they meet—talk about their research and career options.

In panel discussions and in interviews, they also focused on the obstacles that can confront African-American scholars who come from small schools that might not be well known. The opportunity to have such discussions is what makes their annual meeting—and the program itself—vitally important, they said.

"Even though we don't have a large network of African-American students in our respective institutions or departments, when we come together at this meeting, we have a support system because everyone is on the same page and going through the same experience," said A. Nicki Washington, who received her Ph.D. in computer science this spring from North Carolina State University. "It motivates us to continue and it also should be a motivating factor to those coming behind us, to see that there are people who make it, people who succeed."

The David and Lucile Packard Foundation established the program in 1992 to help graduates of Historically Black Colleges and Universities (HBCUs) pursue doctorates in the sciences, engineering, and mathematics. The program awarded 15 scholarships annually, each for $100,000 disbursed over 5 years. The foundation stopped funding new scholars in 2003 and turned management of the program over to AAAS.

Since its inception, 147 fellowships have been awarded. To date, 42 of the scholars have gone on to receive Ph.D.'s and three more are expected to receive Ph.D.'s by December; 56 others remain in the Ph.D. pipeline.

The program is important because recent studies have shown that the U.S. science and engineering labor pool is getting older and that interest in these fields among younger people has waned. In order to keep that labor force globally competitive, many experts say, it will be essential to recruit and cultivate future scientists and engineers from the broadest possible pool of talent.

Currently, African Americans comprise only a small percentage of the students earning advanced degrees in science, technology, and engineering. Averaged over the last three decades, just over one African-American woman per year has received a Ph.D. in physics; in 2004, the scholars program alone produced two such scholars.

"The Graduate Scholars Program shows that there is a lot of talent in HBCUs," said Shirley Malcom, AAAS head of Education and Human Resources. "Though this may not be the prevailing perception in many of the departments in our major research universities, the program indicates that there are many highly qualified students in HBCUs who, with the right financial, moral, and intellectual support, can be extremely successful."

Though the numbers are relatively small, they may provide a foundation for growth, said Marcus Jones, who this year earned his Ph.D. from the Department of Microbiology at New York University School of Medicine.

Generally, a degree obtained from a historically black school "is seen as far inferior compared to a degree from a majority institution," Jones said. But the relationship with the Graduate Scholars Program helps to strengthen the small schools' reputations. And that, Jones added, "will also help with the quantity of minorities entering the sciences and better prepare them for a career in the sciences."

A. Nicki Washington earned her Ph.D. in computer science this year.

Jones, an expert in anthrax, has taken a postdoc position with The Institute for Genomic Research in Rockville, Maryland. Washington has taken a job with Aerospace Corp., based in Chantilly, Virginia.

"It shouldn't necessarily be that we're the first and that we're the elite few," Washington said. "It should be commonplace for African-American students who are in science and engineering, especially at historically black colleges and universities, to be able to go on to obtain higher degrees, so that they can come back and teach at these universities and influence and support and inspire the generations behind them."

MATHEMATICS EDUCATION

AAAS Joins in Efforts to Teach Math Teachers

More than three dozen Washington, D.C., middle-school mathematics teachers are preparing to go back to school—but this fall, they'll be the ones learning. Twenty teachers will join a class of 18 already enrolled in a 3-year professional master's program in middle-school mathematics, a new degree at The George Washington University (GW).

The middle-grades teachers are the most important to target, as research shows that math learning slips during the early teen years, explained GW Math Department Chairman Dan Ullman, who taught a class for the teachers this summer at AAAS. Ullman's class was featured on the 13 July evening news on WRC-TV, the local NBC affiliate for Washington, D.C., Virginia, and Maryland.

Besides teaching their own classes full-time, the teachers meet weekly to practice math and compare teaching methods. They also meet leading national math educators and observe each other's classes. This fall, teachers will begin coaching their colleagues for 9 months, so that every D.C. public, private, and charter school teacher might eventually benefit from the program.

"This [program] gives you an opportunity to constantly be invigorated," said Michelle Johncock, an Edmund Burke School teacher entering her second year of the program. "It doesn't let teachers fall into a rut of teaching."

A cadre of D.C. middle-school science teachers will begin a similar program this fall. In preparation, Jo Ellen Roseman, director of AAAS's Project 2061, which laid the groundwork for the nationwide science standards movement of the 1990s, taught GW science faculty this summer to design a curriculum based on those standards.

AAAS administers the programs in partnership with GW and the D.C. Public School System. Funding is provided by the D.C. State Education Office, through the Mathematics and Science Partnership Program of the U.S. Department of Education.

—Lonnie Shekhtman

Ultrafast Dynamics of Solute-Solvent Complexation Observed at Thermal Equilibrium in Real Time

Junrong Zheng, Kyungwon Kwak, John Asbury,* Xin Chen, Ivan R. Piletic, M. D. Fayer†

In general, the formation and dissociation of solute-solvent complexes have been too rapid to measure without disturbing the thermal equilibrium. We were able to do so with the use of two-dimensional infrared vibrational echo spectroscopy, an ultrafast vibrational analog of two-dimensional nuclear magnetic resonance spectroscopy. The equilibrium dynamics of phenol complexation to benzene in a benzene–carbon tetrachloride solvent mixture were measured in real time by the appearance of off-diagonal peaks in the two-dimensional vibrational echo spectrum of the phenol hydroxyl stretch. The dissociation time constant τ_d for the phenol-benzene complex was 8 picoseconds. Adding two electron-donating methyl groups to the benzene nearly tripled the value of τ_d and stabilized the complex, whereas bromobenzene, with an electron-withdrawing bromo group, formed a slightly weaker complex with a slightly lower τ_d. The spectroscopic method holds promise for studying a wide variety of other fast chemical exchange processes.

Solvents play an enormous role in practical chemistry by influencing the reactivity of dissolved substrates (*1*). In part, the influence stems from polarization effects or nonspecific solute-solvent interactions for which the solvent structure around a solute can be described in terms of an isotropic radial distribution function (*2*). However, specific intermolecular interactions, such as hydrogen bonding, can lead to structurally characterizable solute-solvent complexes that are constantly forming and dissociating under thermal equilibrium conditions on very short time scales (*3*). The dynamics of these transient species can play an important role in the physical and chemical properties of a solute-solvent system by affecting reaction rates, reaction mechanisms, and product ratios (*1*).

If the complexes persist for microseconds or longer, their thermal equilibrium dissociation and exchange dynamics can be studied by means of two-dimensional (2D) nuclear magnetic resonance (NMR) techniques (*4*–*6*). However, for the majority of organic and other types of nonaqueous solutions, in which a vast number of reactions take place, the solute-solvent complexes are bound by energies on the order of a few k_BT (where k_B is the Boltzmann constant and T is absolute temperature; $k_BT \approx 0.6$ kcal/mol at room temperature) and therefore form and dissociate on subnanosecond time scales. As a result, NMR studies cannot distinguish the rapid exchange events and can offer only a dynamically averaged view of the system.

Ultrashort laser pulses have long offered a means of probing rapid dynamics. However, ultrafast absorption and fluorescence techniques have been hindered by the need to perturb the chemical properties of the system in order to study it. These techniques measure net changes in state populations. Thus, electronic excitation can be used to initiate a reaction on a femtosecond time scale by placing a molecule in a higher electronic state, with subsequent fast probing of new product formation, but the system is then no longer observed in its chemical equilibrium state. What is needed to probe equilibrium solvent-solute interactions is an ultrashort analog of 2D NMR spectroscopy. Here, we apply such an analog—ultrafast 2D infrared (2D IR) vibrational echo spectroscopy—to probe the dynamics of phenol complexation with several benzene-based solvents.

Coherent spectroscopy: From NMR to IR. Both the NMR and IR vibrational echo techniques involve pulse sequences that induce and then probe the coherent evolution of excitations (nuclear spins for NMR and vibrations for IR) of a molecular system. The molecules in a given environment (for example, free versus complexed) are induced to "oscillate" in spin states or vibrational states, all at the same time and with the same phase by the first pulse in the sequence. The effect of the first pulse, along with the manipulation of the phase relationships among the vibrational oscillators by the following pulses in the sequence, is an important feature that 2D IR vibrational echo spectroscopy has in common with 2D NMR. The later pulses generate observable signals that are sensitive to change in environments of individual molecules during the experiment (e.g., from free to complexed solute or vice versa), even if there is no change of aggregate populations in the distinct environments (the observable in linear spectroscopy). The critical difference between the IR and NMR variants is that the IR pulse sequence acts on a time scale six orders of magnitude faster than the NMR sequence.

Vibrational echo–based 2D IR experiments have been applied to the study of the intramolecular coupling of vibrational modes (v), the structure of proteins, and the dynamics of hydrogen bonds by observing the positions of peaks or the change in the shape of peaks in the 2D spectrum (*7*–*9*). In the present study, intermolecular chemical exchange causes new peaks in the spectrum to appear and grow, yielding the rate of chemical exchange.

Phenol binds weakly to benzene. Intermolecular chemical exchange under thermal equilibrium conditions is ubiquitous in nature. It forms the basis for supramolecular chemistry (*10*), host-guest chemistry (*10*), chemical and biological recognition (*11*), and self-assembly (*10*). The dynamics of complexes involving noncovalent interactions with aromatic rings are pivotal to the protein-ligand recognition and concomitantly to drug design (*11*). The phenol-benzene complex cuts to the essence of interactions between protic and hydrophobic groups so widespread in proteins and surfactants. More fundamentally, the specific attraction between the polar OH group on phenol and the polarizable π-electron cloud on benzene is an intriguing intermediate case between dispersion forces and hydrogen bonding. The phenol-benzene complex appears to be mainly a van der Waals interaction (*12*), although it is also referred to as π-hydrogen bonding (*11*, *13*) and has recently been found to be of biological importance. π-Hydrogen bonding can stabilize α helices in proteins and plays an important role in cellular and synaptic signal transmission (*13*).

The formation enthalpy for the gas-phase phenol-benzene complex is ~4 kcal/mol (*12*). The gas-phase structure has been determined by electronic structure calculations (*14*). Figure 1 shows two views of the structure of the phenol-benzene complex. In contrast to chemical intuition, the hydroxyl group does not point to the center of the benzene ring, but rather points between adjacent carbons. One of the ortho hydrogens on the phenol also points

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between a pair of carbons that are on the opposite side of the benzene ring. Additional higher level electronic structure calculations performed as part of this study confirm this structure and show that the other complexes studied here have similar structures. Previous studies in liquids demonstrate the existence of the phenol-benzene complex and have measured its enthalpy of formation in CCl_4 (1.56 kcal/mol at room temperature) in temperature-dependent linear IR absorption experiments (*15*) (see below).

We used 2D IR vibrational echo spectroscopy to extract the binding kinetics of phenol-benzene complexes in solution. In addition, we have examined the effect of modifying benzene with electron-donating groups (*p*-xylene or *p*-dimethylbenzene) and an electron-withdrawing group (bromobenzene). Experimental specifics are described below for the benzene studies, with results from the corresponding substituted benzene studies given at the end for comparison.

For the purposes of our experimental study, phenol presents several advantages. The hydroxyl stretch is an isolated vibration with a strong absorption cross section and relatively long vibrational lifetime, so IR excitation is efficient and there is a sufficient time window to observe its dynamics. Moreover, the hydroxyl stretch frequency is sensitive to formation of the complex with benzene. By deuterating the hydroxyl group (OD rather than OH), we transferred the stretching mode away from aromatic C-H stretching bands of similar frequency. The OD stretching band of free phenol in pure CCl_4 is centered at 2665 cm^{-1} (Fig. 2, dotted curve). The spectrum of phenol in pure benzene shows a red-shifted (lower energy) band in this region at 2631 cm^{-1} (Fig. 2, dashed curve), resulting from formation of the phenol-benzene complex. By diluting the benzene with CCl_4, we can shift the equilibrium toward more free phenol. At the concentrations used here in the mixed solution (phenol/benzene/CCl_4 molar ratio 2:40:100, respectively), the absorptions of free and complexed phenol are both prominent in the spectrum (Fig. 2, solid line). Integration of these bands, calibrated from the pure samples, yields the concentrations of complexed and free phenol, which in turn determine the equilibrium constant for complex formation (K_{eq} = [complex]/[phenol][benzene] = 0.26 at 298 K).

To measure the formation and dissociation kinetics of the complex in this solution, we apply three successive IR pulses with the same polarization, which induce the subsequent emission in a distinct direction of a time-delayed fourth pulse—the vibrational echo. The transform-limited pulses (50 fs, <4 cycles of light) are produced using a Ti:sapphire regeneratively amplified laser system coupled to an optical parametric oscillator, and they span sufficient bandwidth (300 cm^{-1} centered at ~4 μm, or 2500 cm^{-1}) to cover the $v = 0$ to $v = 1$ (hereafter denoted 0-1) and 1-2 transitions of the hydroxyl OD stretching modes in both free and complexed phenol. The echo pulse is detected, with frequency and phase resolution, by combining with a fifth (local oscillator) pulse, and the combined pulse is dispersed in a spectrograph. Data are thus obtained as a function of three variables: the emitted echo frequency ω_m (measured directly by the spectrograph), the variable time delay between the first and second pulses (τ), and the variable time delay between the second and third pulses (T_w, the variable "waiting" time). By numerical Fourier transform (FT), the τ scan data taken at each T_w are mapped to a second frequency variable ω_τ. The data are then plotted in three dimensions, showing the amplitude as a function of both ω_τ and ω_m (which correspond to the ω_1 and ω_3 axes, respectively, in 2D NMR). The experimental setup is shown in fig. S1; further experimental details are given in (*16*).

Chemical exchange creates new off-diagonal peaks. Figure 3 displays the 2D IR vibrational echo spectra as contour plots at a very short T_w (200 fs, Fig. 3A) and a long T_w (14 ps, Fig. 3B) (1 fs = 10^{-3} ps = 10^{-15} s). The data have been normalized to the largest peak at each T_w. The red contours are positive-going (0-1 vibrational transition) and the blue contours are negative-going (1-2 vibrational transition). As discussed below, the 0-1 signal comes from two quantum pathways that are related to bleaching of the ground state and stimulated emission, both of which produce a vibrational echo pulse that is in phase with (and therefore adds to) the local oscillator pulse to produce a positive-going signal. The 1-2 signal arises because there is a new absorption that was not present before the first two excitation pulses. The 1-2 vibrational echo pulse is 180° out of phase with (and thus subtracts from) the local oscillator to produce

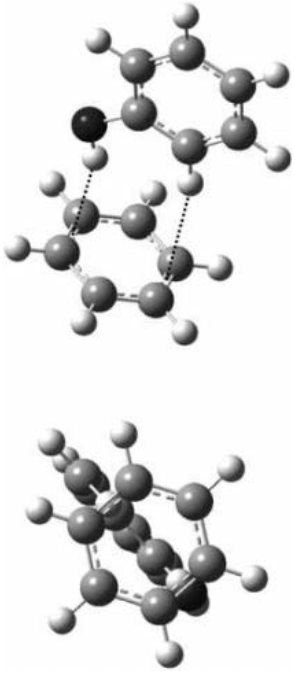


Fig. 1. Two views of the structure of the phenol-benzene complex calculated with DFT at B3LYP/6-31+ G (d,p) level in the gas phase.

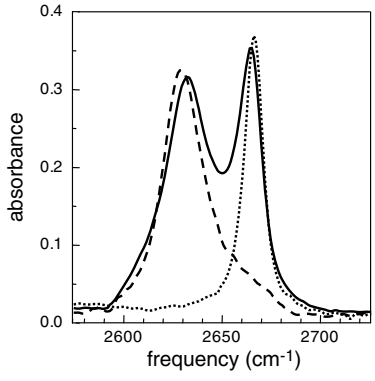


Fig. 2. FT-IR absorption spectra of the OD stretch of phenol-OD (hydroxyl H replaced with D) in CCl_4 (free phenol, dotted curve), phenol in benzene (benzene-phenol complex, dashed curve), and phenol in the mixed benzene-CCl_4 solvent (2:5 molar ratio), which displays absorptions of both free and complexed phenol (solid curve).

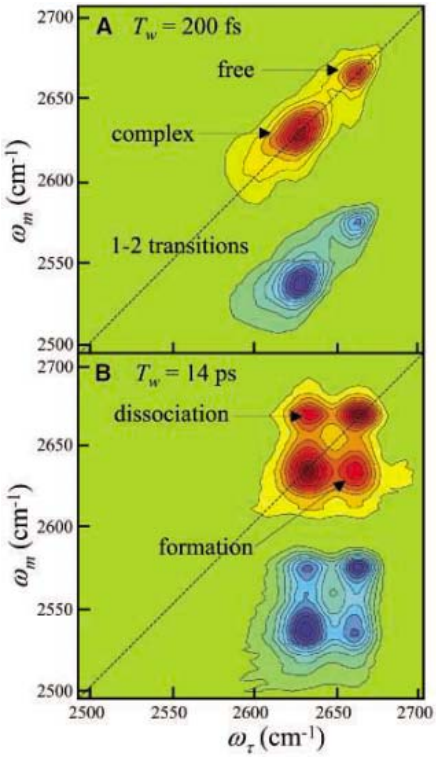


Fig. 3. 2D IR vibrational echo spectra of the OD stretch of phenol in the mixed benzene-CCl_4 solvent. Each contour represents a 10% change. (**A**) Data for T_w = 200 fs. The red contours on the diagonal (positive going) are the 0-1 transitions of the free phenol and the phenol-benzene complex. The blue contours off the diagonal (negative going) are the corresponding 1-2 transition peaks. (**B**) Data for T_w = 14 ps. Additional peaks have grown in because of chemical exchange, that is, the formation and dissociation of the phenol-benzene complex.

a negative-going signal. At T_w = 200 fs, there are two peaks on the diagonal (0-1 transitions) and the corresponding 1-2 transition peaks are off-diagonal. There are no off-diagonal peaks in the 0-1 region because 200 fs is short relative to the exchange time; by contrast, T_w = 14 ps (Fig. 3B) is long relative to the exchange time, and additional peaks have grown in.

The 2D IR vibrational echo spectra in Fig. 3, A and B, can be understood in terms of time-dependent diagrammatic perturbation theory, which describes the nonlinear optical interactions with the molecular vibrations (*17*, *18*). (See fig. S2 for detailed diagrams showing how all the peaks are generated.) The frequency at which the first pulse excites a mode is the mode frequency on the ω_τ axis (horizontal axis): 2631 cm^{-1} for the 0-1 transition in free phenol and 2665 cm^{-1} for the complex. The third pulse induces the vibrational echo pulse, which is emitted after a time delay at the precise frequency of the vibrational transition that interacted with that third pulse. The frequency of the vibrational echo emission is the frequency on the ω_m axis (the vertical axis). In Fig. 3A, the data are taken before chemical exchange. For the 0-1 vibrational transitions, the third pulse induces the vibrational echo emission at the same frequencies excited by the first pulse, so there are two peaks on the diagonal where $\omega_\tau = \omega_m$ (red peaks in Fig. 3A). If the frequency of vibrational echo emission (ω_m, third pulse frequency) is different from the frequency of initial excitation (ω_τ, first pulse frequency), peaks will appear off-diagonal. Again, in Fig. 3A, the blue peaks are off-diagonal by the vibrational anharmonicity (*19*) because the modes are initially excited at their 0-1 frequencies (ω_τ) but the third pulse causes vibrational echo emission at their 1-2 frequencies (ω_m). Even in the absence of chemical exchange, the peaks observed at very short T_w delays (Fig. 3A) will undergo evolution with increasing T_w, because of both spectral diffusion (*17*, *18*, *20*) (see below), which changes the shapes of the peaks, and vibrational lifetime decay and orientational relaxation, which cause the peaks to decay in amplitude.

The influence of chemical exchange on the 2D correlation spectrum can be easily understood in terms of the ideas presented above. If some complexed phenols are liberated during the T_w period, then the third pulse will cause the emission of the vibrational echo at the frequency of the free phenol OD stretch. The frequency of emission ω_m then differs from the excitation frequency ω_τ for these specific molecules. The result will be an off-diagonal peak that appears only if chemical exchange occurs. Because the free phenol absorbs at higher frequency than complexed phenol, this off-diagonal peak is shifted to higher frequency along the ω_m axis by the frequency difference (34 cm^{-1}) between the free and complexed modes. Conversely, if some free phenols bind to benzene during the T_w period, then the third pulse will produce an off-diagonal peak for these (formerly) free phenols, which is shifted to lower frequency along the ω_m axis by the same amount. Identical considerations apply for both the 0-1 and 1-2 regions of the spectrum. This behavior is shown in Fig. 3B, wherein substantial chemical exchange has led to the generation of a block of four red peaks and a block of four blue peaks; the two new peaks in each block were not present at T_w = 200 fs. Clearly some complexes have dissociated and others have formed. The growth of the additional off-diagonal peaks with increasing T_w is directly related to the time dependence of the chemical exchange.

The phenol-benzene complex persists for 8 ps. Figure 4 displays the time evolution of the correlation spectrum in the 0-1 transition region as 3D representations. (More plots are shown in fig. S3.) The data have been normalized to the largest peak at each T_w. As in Fig. 3, at T_w = 200 fs there are only diagonal peaks. The peak in the foreground is the vibration echo of complexed phenol. At T_w = 2 ps, two changes are evident. First, the shapes of the diagonal peaks have altered. Whereas at T_w = 200 fs the peaks are elongated along the diagonal, by 2 ps they have become symmetrically rounded. The change in shape is caused by spectral diffusion. At short times, the vibrational transitions are inhomogeneously broadened. As time proceeds, the fluctuations in the solvent environment cause the transition frequency to sample all possible values, and each line becomes dynamically broadened. The change in shape provides information on the dynamic solute-solvent interactions, but here we focus our analysis exclusively on the chemical exchange. At 2 ps, the off-diagonal peaks are just becoming visible. By 5 ps, the off-diagonal peaks are clearly evident, and they continue to grow in amplitude, as shown in the 10-ps and 14-ps plots.

Simple inspection of the data reveals that the phenol-benzene complexes form and dissociate on a picosecond time scale. To obtain quantitative rates, we fit the data with the use of time-dependent diagrammatic perturbation theory and kinetic equations to describe the exchange dynamics. Because of spectral diffusion, the shapes of the peaks change. In the absence of all other dynamical processes, the change in shape preserves the volume of a peak, but the peak amplitude is reduced as the peak broadens along the ω_τ axis. Therefore, the integrated peak volumes are fit to obtain the population dynamics. There are three processes that contribute to the change in the peak volumes: the OD vibrational lifetime relaxation in free and complexed phenol (lifetimes T_1^f and T_1^c), the orientational relaxation (time constants τ_r^f and τ_r^c), and the chemical exchange (dissociation and formation rate constants k_d and k_f). The kinetic scheme is shown in Fig. 5A; more details are described in fig. S4. The vibrational relaxation and orientational relaxation lead to diminishing intensities of all peaks with increasing time. Even complete orientational randomization during the T_w period does not cause the vibrational echo to decay to zero. Therefore, the chemical exchange time need not be short relative to the orientational relaxation time. The chemical exchange causes the diagonal peaks to diminish and the off-diagonal peaks to grow, as can be seen in Figs. 3 and 4. The dissociation rate (number per unit time) of the complex equals the formation rate if the system is in equilibrium (see below). The complex dissociation time constant τ_d is independent of concentration and is therefore used here ($\tau_d = 1/k_d$, where k_d is the dissociation rate constant; the dissociation rate is k_d[complex]).

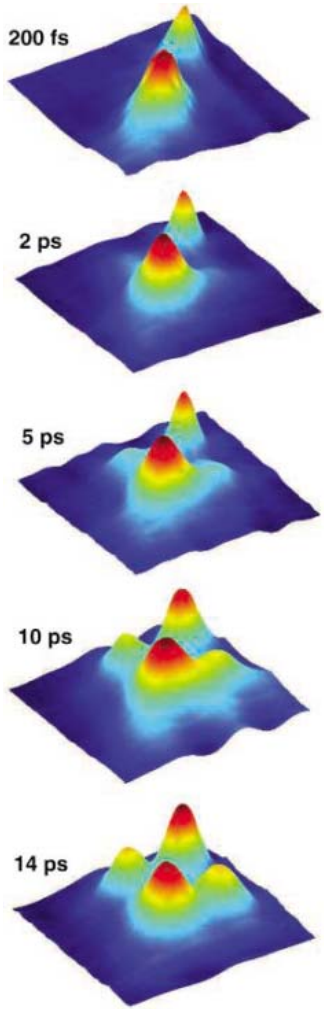


Fig. 4. The time dependence of the 2D IR vibrational echo spectrum in the 0-1 transition region. As T_w increases, the off-diagonal peaks grow in because of chemical exchange (formation and dissociation of the benzene-phenol complex). Between 200 fs and 2 ps, the peaks change shape because of spectral diffusion. Inspection of the data shows a picosecond time scale for the chemical exchange (growth of the off-diagonal peaks).

The data for the 0-1 transition region consist of four time-dependent components: the two diagonal peaks (complexed and free phenol) and the two off-diagonal peaks (dissociation and formation of complexes during the experiment). A similar treatment is applied for the 1-2 region below. All four peaks can be reproduced with the single fitting parameter τ_d. The input parameters used are $T_1^c = 10$ ps, $T_1^f = 12.5$ ps, $\tau_r^c = 3.4$ ps, $\tau_r^f = 2.9$ ps, and the ratio of the complexed and free phenol concentrations [complex]/[free] = 0.8. The lifetimes and the orientational relaxation times were measured with polarization-selective IR pump-probe experiments (*21*) on the OD stretch of the complex in pure benzene solvent and free phenol in pure CCl_4 solvent. The orientational relaxation time constants were corrected for the small viscosity difference between the pure solvents and mixed solvents. The concentration ratio was measured with linear absorption FT-IR spectroscopy.

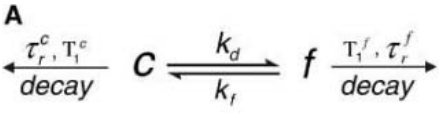
Figure 5B shows the peak volume data for the 0-1 transition region of the spectrum as a function of T_w. The fits (solid curves) to the time dependence of all of the peaks using a single adjustable parameter, τ_d, are very good. From the fits, dissociation of the complex proceeds with a time constant $\tau_d = 8 \pm 2$ ps. The error bars obtained from the single-parameter fits are smaller than 2 ps. The cited error bars mainly arise from the uncertainties in all of the parameters in the calculation, rather than the quality of the fit.

Vibrational excitation does not perturb the equilibrium. An important aspect of 2D NMR is that the manipulation of spin states by a pulse sequence produces a negligible perturbation of the molecular system, and therefore the experiment does not move the system away from equilibrium. It is possible to determine whether vibrational excitation in the current system shifts the concentrations away from their equilibrium values or changes τ_d. Even if vibrational excitation does shift the system out of equilibrium, the dynamics for the equilibrium system can nonetheless be extracted. If the thermal equilibrium is not perturbed by vibrational excitation, both off-diagonal peaks (formation and dissociation of the complex) should have the same time dependence. In the 0-1 transition region, the integrated off-diagonal intensities (circles and squares in Fig. 5B) do show matching growth rates as a function of T_w, confirming an undisturbed equilibrium. In other words, the pulse sequence has no impact on the aggregate populations of free and bound phenol in solution. For each complex formed during the T_w period, another complex liberates a phenol.

It is possible that vibrational excitation changes both the dissociation rate and the complex formation rate. If both rates were changed to the same extent, the equilibrium concentrations would be unchanged and the off-diagonal peaks would grow in at the same rate, as observed. It is possible to test whether vibrational excitation changes the dissociation time constant τ_d directly and conclusively. Of more importance, it is possible to see how the equilibrium exchange time constants can be extracted even if vibrational excitation does change the dynamics of dissociation and formation of complexes.

A series of qualitative energy level/kinetic diagrams (Fig. 6) clarifies how to do this and enables a more detailed understanding of the 2D vibrational echo measurements of chemical exchange. These diagrams are not rigorous quantum mechanical diagrams as used in diagrammatic perturbation theory, but their simplicity has important heuristic value. (The full set of double-sided Feynman diagrams pertaining to this problem is given in fig. S2.) The diagrams show how the off-diagonal peaks for the dissociation of complexed phenol to free phenol ($c \rightarrow f$) are generated in both the 0-1 (Fig. 6, A and B) and the 1-2 (Fig. 6C) portions of the 2D spectrum. These are the peaks at $\omega_\tau = 2631$ cm^{-1} and $\omega_m = 2665$ cm^{-1} (0-1), and at $\omega_\tau = 2631$ cm^{-1} and $\omega_m = 2575$ cm^{-1} (1-2) in Fig. 3B. The other off-diagonal peaks, representing complex formation, arise in the same manner.

The signal for the off-diagonal 0-1 portion $c \rightarrow f$ peak has two contributions illustrated in Fig. 6, A and B. In Fig. 6A, the arrow representing pulse 1 makes a coherent superposition state of the $v = 0$ and $v = 1$ levels of the phenol-benzene complexes at frequency



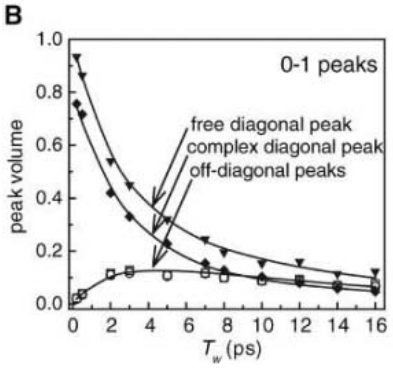


Fig. 5. (**A**) Kinetic scheme. The complexed (c) and free (f) phenols can exchange with dissociation rate constant k_d and formation rate constant k_f. The exchange process causes the off-diagonal peaks to grow in and the diagonal peaks to diminish. All of the peaks decay because of vibrational relaxation with lifetime T_1^i and rotational relaxation with time constant τ_r^i. (**B**) Peak volume data (scaled with extinction coefficient differences; see fig. S4) and fits to the data. There is one adjustable parameter to fit all of the data: the dissociation time constant, τ_d, of the phenol-benzene complex. Fits to the data for the four peaks in the 0-1 transition region give $\tau_d = 8$ ps. The two off-diagonal peaks (circles and squares) grow in at the same rate, showing that the thermal equilibrium is not perturbed by vibrational excitation to the first vibrationally excited state of the hydroxyl stretch.

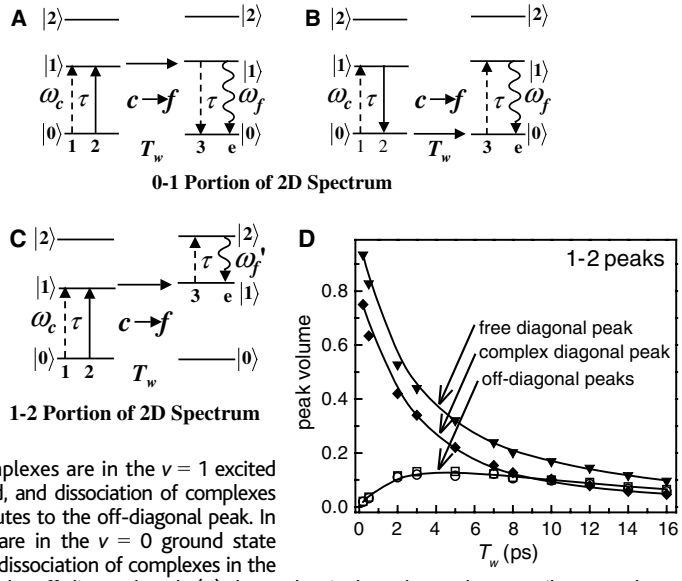


Fig. 6. (**A** to **C**) Energy level/kinetic diagrams that show the pulse sequence pathways that give rise to the vibrational echo signal for the off-diagonal peaks created by the dissociation of the phenol benzene complex in the 0-1 and 1-2 portions of the 2D vibrational echo spectrum (Fig. 3B). (A) and (B) show the two pathways for the 0-1 portion of the spectrum. Each pathway contributes half of the 0-1 dissociation off-diagonal peak. In pathway (A), complexes are in the $v = 1$ excited state during the T_w period, and dissociation of complexes in the $v = 1$ state contributes to the off-diagonal peak. In pathway (B), complexes are in the $v = 0$ ground state during the T_w period, and dissociation of complexes in the $v = 0$ state contributes to the off-diagonal peak. (C) shows the single pathway that contributes to the 0-1 dissociation off-diagonal peak in the 1-2 portion of the spectrum. In pathway (C), complexes are in the $v = 1$ excited state during the T_w period. Only complexes that dissociate while in the excited state contribute to the dissociation off-diagonal peak in the 1-2 portion of the spectrum. The difference between the two contributions to the signal for the 0-1 portion and the single contribution for the 1-2 portion permit the extraction of the equilibrium dissociation time even if vibrational excitation changes the dissociation and formation rate constants from their equilibrium values (see text). (**D**) Data and calculated curves for the 1-2 portion of the spectrum (blue peaks in Fig. 3B) give the same τ_d value as the 0-1 portion, demonstrating that excitation of the hydroxyl stretch does not influence the dissociation rate constant.

ω_c. In all diagrams, a dashed arrow represents the creation of a coherent superposition state. A coherent superposition state is equivalent to an in-plane precessing magnetization that is generated by the first pulse in a 2D NMR pulse sequence. During the τ period between pulses 1 and 2, exchange does not contribute to the growth of the off-diagonal peak, because the exchange time τ_d is long relative to the inverse of the frequency difference between the *c* and *f* peaks. Therefore, jumps from *c* to *f* will produce an ensemble of superposition states in *f* with random phase, resulting in no contribution to the off-diagonal peak signal. After time τ, the second pulse generates a population in the excited $v = 1$ state. A solid arrow in all diagrams represents the generation of a population. Some of the molecules that are initially complexes have now been "labeled" in the $v = 1$ state. If some of these complexes dissociate ($c \rightarrow f$) during the period T_w, pulse 3 will produce a coherent superposition state (dashed arrow in Fig. 6A) oscillating at frequency ω_f. The vibrational echo pulse will be emitted (wavy arrow) at frequency ω_f. Therefore, the ω_τ frequency is ω_c, but the ω_m frequency is ω_f. The $c \rightarrow f$ off-diagonal peak is produced. The pathway in Fig. 6A for which the dissociation occurs with molecules labeled in $v = 1$ accounts for half of the $c \rightarrow f$ off-diagonal peak signal in the 0-1 portion of the spectrum.

The pathway shown in Fig. 6B accounts for the other half of the $c \rightarrow f$ off-diagonal peak signal. Again, pulse 1 produces a 0-1 coherent superposition state (dashed arrow), but pulse 2 produces a population (solid arrow) in the $v = 0$ state instead of in the $v = 1$ state, again labeling molecules that are initially complexes. This pathway involves molecules labeled in the $v = 0$ state during the T_w period. For those complexes that undergo dissociation during the T_w period, pulse 3 will produce a coherent superposition state (dashed arrow) at frequency ω_f, and the vibrational echo (wavy arrow) will be emitted at ω_f. As in the path shown in Fig. 6A, in Fig. 6B the ω_τ frequency is ω_c, but the ω_m frequency is ω_f, and this path contributes to the $c \rightarrow f$ off-diagonal peak. For both pathways, pulse 3 produces a coherent superposition state. In NMR, this coherent superposition state yields an in-plane precessing magnetization (a macroscopic oscillating magnetic dipole moment) that is detected with a pickup coil. In the vibrational experiment, the coherent superposition state in each pathway gives rise to a macroscopic oscillating electric dipole moment that emits light, the vibrational echo pulse (wavy arrows).

The important point to see from Fig. 6, A and B, is that half of the signal comes from molecules that are in their $v = 1$ state during the T_w period and half comes from molecules in the $v = 0$ state. If vibrational excitation influences the equilibrium dynamics, then the time evolution of the exchange off-diagonal peaks will be affected and τ_d can be altered from its equilibrium value. Whether τ_d is changed by vibrational excitation can be tested; if it is, then its equilibrium value can be recovered by examining the 1-2 portion of the 2D vibrational echo spectrum in addition to the 0-1 portion. Figure 6C shows the single diagram that contributes to the $c \rightarrow f$ off-diagonal peak in the 1-2 portion of the spectrum. In Fig. 6C, pulse 1 creates a 0-1 coherent superposition state (dashed arrow) and pulse 2 generates a population (solid arrow) of complexed phenols in the $v = 1$ state. There is only one path because it is necessary to have molecules in the $v = 1$ state so that pulse 3 can produce a coherent superposition state (dashed arrow) of $v = 1$ and $v = 2$, which is then followed by vibrational echo emission at the 1-2 frequency (blue peaks in Fig. 3B). If dissociation of complexed phenols occurs during the T_w period, the blue $c \rightarrow f$ off-diagonal peak is generated. Thus, the entire signal for this off-diagonal peak arises solely from complexes that were in the $v = 1$ state and underwent dissociation during the T_w period.

This property does not hold for the red $c \rightarrow f$ off-diagonal peak, for which half of the signal is generated by molecules that were in the $v = 1$ state and half by molecules in the $v = 0$ state during the T_w period. If excitation to the vibrationally excited $v = 1$ state changes the equilibrium dynamics, then the τ_d determined from the 0-1 portion of the spectrum will be different from the τ_d determined from the 1-2 portion of the spectrum. The τ_d value determined from the 0-1 portion of the spectrum is an average of its ground state and first vibrationally excited state value. Using the two values of τ_d determined from the 0-1 and 1-2 portions of the 2D vibrational echo spectrum, the value for the ground state (unperturbed thermal equilibrium) pathway can be easily obtained. If the two values are the same, then vibrational excitation did not have an experimentally measurable influence on the equilibrium dynamics of the system.

Figure 6D shows the data and calculated curves for the 1-2 region of the 2D vibrational echo spectrum. There are no adjustable parameters. The data are reproduced with the identical parameters used for the 0-1 region, including $\tau_d = 8$ ps. Variation of τ_d does not improve the agreement. Thus, within experimental uncertainty, for the phenol-benzene complex and the other systems studied here, exciting the hydroxyl stretch has no influence on the dissociation time and, as discussed above, vibrational excitation does not change the equilibrium constant.

Electron-rich benzene derivatives make stronger complexes. In addition to the phenol-benzene complex, the phenol-bromobenzene and phenol–*p*-xylene complexes were studied in the identical manner. The dissociation time for the phenol–*p*-xylene complex is considerably slower than for the phenol-benzene complex, with $\tau_d = 21 \pm 3$ ps. The dissociation time constant for the phenol-bromobenzene complex is $\tau_d = 6 \pm 3$ ps. This value is within experimental uncertainty of the phenol-benzene complex τ_d. However, the error bars are determined in large part by the errors of all of the parameters used in the calculations, rather than by the quality of the fits. A direct comparison for the phenol-benzene and phenol-bromobenzene complexes (at, for example, 7 ps) of the sizes of their diagonal peaks versus their respective dissociation-induced off-diagonal peaks clearly shows that the bromobenzene complex dissociates more rapidly.

To investigate the trend in the τ_d values, we measured the temperature dependences of the complexation equilibria for all three systems by IR absorption and then used these values to determine the bond enthalpies, ΔH^0, of the complexes. (Here, the standard solvent concentrations are defined to be the concentrations used in the experiments.) The ΔH^0 values extracted from van't Hoff plots were –1.21 kcal/mol for the phenol-bromobenzene complex, –1.67 kcal/mol for the phenol-benzene complex, and –2.23 kcal/mol for the phenol–*p*-xylene complex (fig. S5). Thus, as the bond enthalpy increases (stronger bond), the dissociation time also increases. If the free energy of activation for dissociation ($\Delta G^\ddagger$) scales with the bond enthalpies, we can qualitatively understand the trend in τ_d. The electron-withdrawing bromine weakens the complex by reducing the π-electron density of the ring, leading to a faster dissociation time. Conversely, in *p*-xylene, the electron-donating methyl groups increase the π-electron density of the ring, resulting in a stronger complex and correspondingly longer dissociation time.

The 2D IR vibrational echo technique is general. The method used here is general for measuring fast chemical exchange dynamics in the ground electronic state, and appears promising for studies of a wide range of molecular isomerizations and electron and proton transfer processes under thermal equilibrium conditions. There are three conditions necessary to apply this technique to study thermal equilibrium exchange phenomena: (i) There must be at least one IR active mode that has a distinct frequency for each species undergoing exchange, (ii) the concentrations of all the equilibrated species must be high enough for detection, and (iii) the exchange rate must be comparable to or shorter than the vibrational lifetime of the vibration that is being used as the probe. It is important to note that the vibrational mode that is used as the probe need not be directly involved in the exchange process, as is the hydroxyl stretch used in this study. It is sufficient that the exchange causes the mode frequency to change.

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REPORTS

Magnetic Field–Induced Superconductivity in the Ferromagnet URhGe

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In several metals, including URhGe, superconductivity has recently been observed to appear and coexist with ferromagnetism at temperatures well below that at which the ferromagnetic state forms. However, the material characteristics leading to such a state of coexistence have not yet been fully elucidated. We report that in URhGe there is a magnetic transition where the direction of the spin axis changes when a magnetic field of 12 tesla is applied parallel to the crystal *b* axis. We also report that a second pocket of superconductivity occurs at low temperature for a range of fields enveloping this magnetic transition, well above the field of 2 tesla at which superconductivity is first destroyed. Our findings strongly suggest that excitations in which the spins rotate stimulate superconductivity in the neighborhood of a quantum phase transition under high magnetic field.

The discovery of fundamentally new correlated electronic phases is rare in condensed matter physics. A promising parameter region in which to prospect for the emergence of such states is, however, found when a material is tuned through a continuous magnetic phase transition at very low temperature. As a material is tuned through such a transition, magnetic fluctuations become soft in energy and have large quantum amplitudes. Under such circumstances it becomes easier to deform the electronic spin system to adopt new ground state configurations potentially brought about by the large amplitudes of the fluctuations themselves. This description appears to apply at the pressures where antiferromagnetic order is suppressed in $CeIn_3$ and $CePd_2Si_2$; a new superconducting phase is induced in a pocket of pressures at low temperature surrounding the critical pressure at which the antiferromagnetism is destroyed (*1*). For a magnetic transition between two different itinerant ferromagnetic phases, the correlated states that can emerge are potentially quite different. Equal spins as opposed to opposite spins must be paired for superconductivity to occur, owing to the magnetic polarization of the electronic band structure. UGe_2 (*2*) and URhGe (*3*) have already attracted attention because of the recent discoveries that bulk superconductivity coexists with ferromagnetism in these materials well below their ferromagnetic transition temperatures. The inference that equal spins are paired in the superconducting state is additionally supported for URhGe by measurements of the critical field necessary to suppress the superconductivity (*4*). We report here that in URhGe a magnetic transition can be induced by applying a much larger magnetic field than that at which superconductivity is first destroyed and that at low temperature a new pocket of superconductivity emerges surrounding this transition.

URhGe is ferromagnetic below a Curie temperature, T_C, of 9.5 K, with a spontaneous moment aligned to the *c* axis of its orthorhombic crystal structure. The field-induced magnetization is smaller for fields applied along the *a* axis than the other crystal axes. We therefore restrict the discussion to fields in the *bc* plane. The first indication that a magnetic transition occurs under magnetic field in URhGe can be found in (*5*), where a jump in the field-induced magnetization was seen at high field and low temperature. We find that such a jump occurs when the field is applied close to the *b* axis direction. We show below that this transition corresponds to a sudden rotation of the moment in the *bc* plane.

Measurements of the torque acting on a single crystal of URhGe (Fig. 1A) show that the component of the magnetization parallel to the *c* axis, M_c, collapses abruptly to zero when a magnetic field of magnitude, H_R, is applied along the *b* axis, with $\mu_0 H_R = 11.7$ T (μ_0 is the permeability of a vacuum). Measurements, both directly in a magnetometer and by neutron scattering (Fig. 1B), show that the magnetization parallel to the *b* axis also increases suddenly at this field, but that the total moment above H_R is close to the value extrapolated from fields well below H_R. Therefore, the main change in the magnetization across the transition is a rotation of the moment toward the *b* axis. For fields aligned at an angle, θ, from the *b* axis, a rapid rotation of the moment occurs at higher fields, $H_R(\theta)$. The moment no longer aligns perfectly to the field direction immediately above the transition, and the transition becomes progressively broader.

In the normal state, there is a clear peak in the electrical resisitivity as a function of the ap-

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plied field close to $H_R(\theta)$ (Fig. 1C). The peak in the resistivity can be described as the sum of two parts: (i) a very narrow δ-function-like peak that is almost temperature-independent (in the temperature range from just above the superconducting transition up to 0.8 K) and (ii) a broader asymmetric peak whose amplitude increases with temperature. The former feature has a width of 0.5 T whereas the latter has a characteristic width of about 5 T. The amplitude of the δ function is smaller for larger θ, and it is completely absent for $\theta \geq 5°$. It follows from its temperature independence that it represents an enhancement of the residual normal-state resistivity. This might be caused by the presence of a finely divided domain structure close to a first-order transition. The amplitude of the broader peak, (ii), depends only weakly on θ. Its field dependence is different from the field dependence of the residual normal-state resistivity; the two can be compared directly in a low quality sample that does not become superconducting. It therefore corresponds in part to an increase in the dynamic scattering rate of the conduction electrons close to $H_R(\theta)$ and is not due simply to a change in the electronic density of states. A low-temperature phase diagram for fields in the *bc* plane that describes all the above results is shown schematically in Fig. 2B. A discontinuous change in the moment orientation occurs across the thick line in the figure. The change becomes continuous at the point where the line ends. In the limit of zero temperature, this point is referred to as a quantum critical end point (QCEP).

In good quality samples with a small normal-state residual resistivity, ρ_0, equivalent to a large residual resistivity ratio (RRR, the resistance at 300 K divided by ρ_0), superconductivity occurs below 2 T as previously reported (*3*). Our finding is that superconductivity reappears over a wide range of fields about H_R below 400 mK. A lower quality sample with RRR = 5, however, was not superconducting at any field, thus suggesting superconductivity in both field ranges is unconventional; that is, the phase of the superconducting order parameter has an intrinsic directional dependence. Measurements on a high-quality single crystal of RRR ≈ 50 are shown in Figs. 1 to 3. The maximum transition temperature, T_s, in the high-field superconducting state is almost 50% greater than T_s observed in zero field. The large field range over which superconductivity occurs around H_R shows that superconductivity exists in regions in which the sample is monodomain and that superconductivity is not confined to domain walls.

Field-induced superconductivity has been observed before in other materials, for example in the Chevrel phase compound $Eu_{3/4}Sn_{1/4}Mo_6S_{7.2}Se_{0.8}$ (*6*) and more recently in several organic superconductors including k-$(BETS)_2FeBr_4$ (*7*) and k-$(BETS)_2FeCl_4$ [in the latter, field induced superconductivity is observed when the field is accurately aligned to two-dimensional (2D) structural planes (*8*, *9*)]. These cases can be explained by a compensation of the applied field by an internal field produced by the polarization of magnetic ions, resulting in a total effective field that is actually small in high applied fields [the Jaccarino-Peter effect (*10*)]. For all these materials the induced moment is parallel to the applied field. In contrast, for URhGe superconductivity occurs when the ordered moment is inclined over a range of angles from 30° to 55° to the applied field direction. It is unlikely that an exchange field due to magnetic moments could completely cancel the effect of the applied field over such a wide range of angles. Further, in the Chevrel phase and organic superconductors the field induced superconductivity is distinct from other phase transitions seen at different fields. The originality of the behavior observed in URhGe is that the maximum T_s occurs exactly at the transition field H_R (Fig. 3). The correlation between superconductivity and the moment rotation transition is confirmed by comparing the dependence of $H_R(\theta)$ with the angle dependence of the superconducting phase boundary (Fig. 2). The superconductivity at high field is thus intimately connected with the moment rotation.

Theoretically, in a ferromagnet, the orientation of the magnetic moment, $\vec{M}$, in a magnetic field of induction, $\vec{B}$, is determined from the balance between the magnetic energy $-\vec{M}\cdot\vec{B}$, which seeks to align the magnetic moment parallel to the field, and the energy required to rotate the moment away from its preferred orientation with respect to the crystal structure. For a fixed direction of the applied magnetic field, the moment orientation usually changes in a continuous manner as the magnitude of the applied field is changed, but a first order process in which the moment rotates discontinuously at some field is possible. It can occur for appropriate values of the crystal field anisotropy and spin-orbit coupling with a fixed magnitude of the magnetic moment. For URhGe the magnitude of the moment depends on the field, and a more complete description is given by a Landau expansion of the free energy, $F = a_x M_x^2 + a_y M_y^2 + (b_x M_x^2 + b_y M_y^2)^2 + b_{xy} M_x^2 M_y^2 - \vec{M}\cdot\vec{B}$. A first-order transition occurs in this case when the term b_{xy} is greater

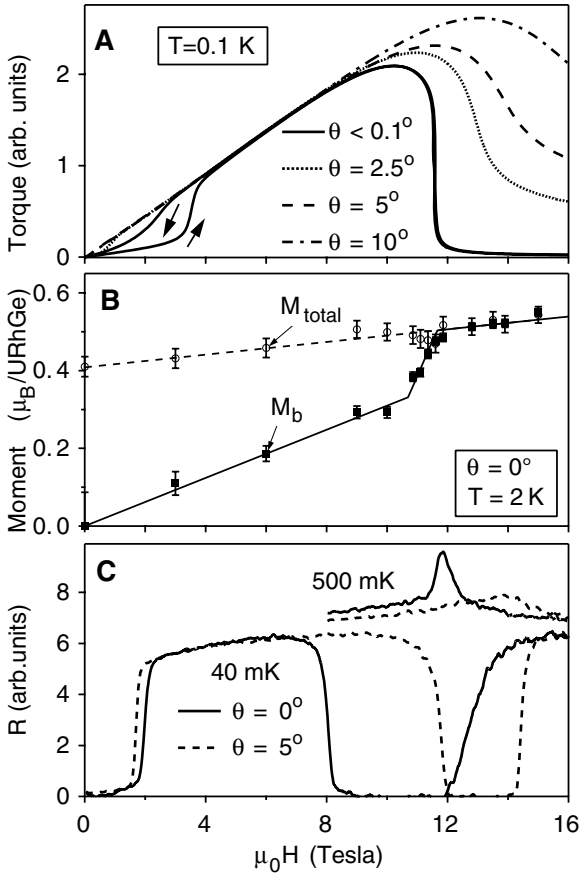

Fig. 1. Field-induced moment rotation and superconductivity in URhGe. (**A**) The magnetic field dependence of the torque acting on a single crystal of URhGe at low temperature (0.1 K). This is shown for different angles, θ, of the field from the crystal *b* axis in the *bc* plane. The arrows indicate measurements made increasing and decreasing the field for $\theta < 0.1°$. In zero field the spontaneous magnetization is parallel to the *c* axis. When θ is close to zero, hysteresis is seen at low fields due to the polarization of the ferromagnetic domain structure by the small field component parallel to the *c* axis. Only a very slight rotation of the field in the *bc* plane is necessary for this component to exceed the coercive field necessary to drive the sample monodomain. The torque, τ, divided by $\mu_0 H$ is then proportional to the saturated ferromagnetic moment perpendicular to the applied field. The sudden drop in the torque at high field corresponds to a sudden reduction of this component of the magnetization. (**B**) The total magnetic moment and the component of the moment parallel to the *b* axis (in units of Bohr magnetons per URhGe) for θ = 0, measured by neutron scattering at 2 K (the lines are to guide the eye) (*23*). The moment parallel to *b* increases rapidly at H_R (the slightly smaller value of H_R and broader transition compared with the torque data are explained by the larger temperature). Error bars show the estimated standard deviation of each measurement. (**C**) The electrical resistance of the sample over the same field range at temperatures of 40 mK and 500 mK. At 500 mK the sample is in the normal state and a clear peak in the resistance is seen at H_R. At 40 mK the resistance is zero for a range of fields about H_R. This pocket of field induced superconductivity occurs in addition to that observed below 2 T [reported previously (*3*, *4*)].

than a critical value (determined by the values of the other coefficients, a_x, a_y, b_x, and b_y). For URhGe the various coefficients can be determined from the initial differential susceptibility parallel to the b axis and Arrott plots of the magnetization for fields parallel to the c axis and for fields $H > H_R$ parallel to the b axis. The condition for a first order transition for fields close to the b axis is found to be satisfied, and the computed phase diagram based on the above expression for the free energy is qualitatively compatible with that shown in Fig. 2B.

The theory of superconductivity mediated by the exchange of spin fluctuations is most often considered close to a ferromagnetic-paramagnetic quantum critical point where the longitudinal differential susceptibility diverges at low energy and wave vectors. Only this region of energy–wave vector space then has to be considered (*11*, *12*). Under these conditions, a large value of the uniform differential susceptibility parallel to the magnetization favors the formation of Cooper pairs with equal spins, whereas a large value of the differential susceptibility perpendicular to the magnetization breaks such pairs. The situation is modified well inside the ferromagnetic state, because the transverse excitations no longer have the same form; they are collective spin waves rather than incoherent overdamped modes. In an isotropic ferromagnet, they can lead to an enhancement of the longitudinal susceptibility due to mode coupling that outweighs their pair-breaking effect (*13*). For URhGe this same mechanism could be active in a modified form. An important aspect not considered in previous theory is the anisotropy of the spin fluctuation spectrum for different directions of the wave-vector transfer, $\vec{q}$. For example, a magnetic-field energy is incurred when $\vec{q}$ is not perpendicular to the change in magnetization associated with an excitation (*14*). For spin rotation excitations in the bc plane, this energy would be absent for wave-vector transfers along the a axis, and excitations propagating in this direction would consequently have a lower energy than along other directions of $\vec{q}$. This could favor a polar superconducting order parameter oriented along the a axis. It is noteworthy that such a state can explain the critical field of the low field superconductivity (*4*). Theoretically, the symmetry of such a state would also be consistent with the crystal structure and ferromagnetism with the moments aligned along the b axis (*15*).

Over recent years, the application of a magnetic field at very low temperature has been established to be an effective tuning parameter to drive a number of materials to a quantum critical point or QCEP [examples are $YbRh_2Si_2$ (*16*), $Sr_3Ru_2O_7$ (*17*), and URu_2Si_2 (*18*)]. In the limit of zero temperature, the divergence of the differential magnetic susceptibility at this point implies a diverging amplitude for zero-point motion (quantum fluctuations) that can destabilize the system relative to other forms of order (*19*). The behavior in URhGe can be compared with that of the almost-2D material $Sr_3Ru_2O_7$, where a new as-yet incompletely identified ground state appears in high quality samples enveloping a QCEP at 7.8 T. For $Sr_3Ru_2O_7$ it has been argued that superconductivity is not viable because of the large field at which the QCEP occurs (*17*). For opposite-spin pairing both paramagnetic limitation and orbital limitation restrict the maximum field up to which superconductivity can survive. For equal-spin pairing only the second limit applies. This requires that the superconducting coherence length, ξ_0, is small enough to satisfy the relation $\phi_0/(2\pi\xi_0^2) > B$ (ϕ_0 is the flux quantum and B the magnetic induction); a value $\xi_0 < 50$ Å would be compatible with the high field superconducting phase of URhGe.

It appears that the high field superconductivity in URhGe, like the superconductivity at low field in UGe_2, is not directly driven by fluctuations associated with a quantum critical point or QCEP separating ferromagnetism from paramagnetism. In both materials superconductivity is instead associated with a magnetic transition between two strongly polarized states, although the transitions differ; in URhGe there is a large change in the transverse moment at the transition, whereas in UGe_2 only the lon-

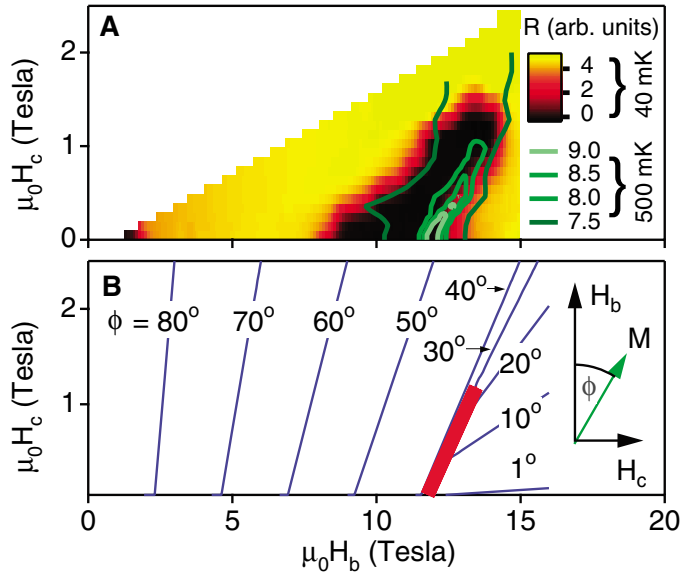

Fig. 2. The low temperature resistivity and magnetic phase diagram for fields in the crystallographic bc plane. (**A**) The measured resistance for fields in the bc plane. The resistance at 40 mK is represented by the color (top scale). The black areas are regions where the sample has zero resistance and is superconducting. Contour lines depict the resistance at 500 mK (bottom scale). The area where superconductivity occurs at low temperature is seen to correspond to the region over which the resistance is peaked at higher temperature. (**B**) A representation of the magnetic phase diagram at low temperature. The thin lines are contours of constant angle, ϕ, of the magnetic moment from the b axis. The thick line denotes a first order transition across which ϕ changes discontinuously. The first order line ends at a QCEP. Beyond this point a sharp crossover behavior still occurs in the field dependence of the moment orientation. The definition of ϕ is illustrated in the sketch at the right, with arrows depicting the direction of the magnetization, M, and of the components of the applied field, H_b and H_c.

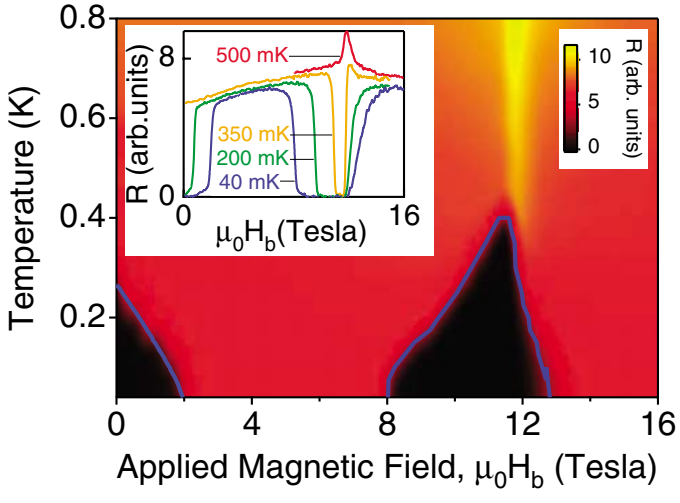

Fig. 3. The field-temperature phase diagram for applied fields parallel to the b axis. The color represents the resistivity. Superconductivity occurs throughout the black region where the resistivity is zero. The maximum transition temperature corresponds to the field, H_R, at which the resistivity has a sharp maximum at higher temperature. The blue solid lines show the position at which the resistance is half its normal-state value (for the data at low field, this was determined more precisely in separate measurements). (Inset) The resistance as a function of field at several temperatures corresponding to horizontal cuts through the main figure.

gitudinal moment changes. For UGe_2 the superconducting coupling strength and transition temperature increase as the magnetic transition is approached by tuning the pressure (*20*). The magnetic transition is, however, first order (*21*), and UGe_2 has not yet been studied under the conditions necessary to drive it to a QCEP. The apparent relationship of high field superconductivity to a field-induced quantum critical point in URhGe established here, however, reinforces the general notion that new strongly correlated electron ground states emerge close to quantum critical transitions between apparently simpler magnetic phases. An interesting possibility is that the low field superconductivity in URhGe might also be related to the same quantum critical point that we now outline. Superconductivity occurs when the upper critical field for the superconducting state, H_{c2}, exceeds the total magnetic field acting on the electrons. For URhGe, as the applied field is reduced from H_R moving the material away from the QCEP, H_{c2} is expected to fall rapidly. Superconductivity would disappear when H_{c2} falls below the applied field (for simplicity, the small internal field in the sample due to its magnetization, $\mu_0 M \approx 0.1$ T, can be ignored). However, if H_{c2} is still finite at low fields, the condition for superconductivity (with a much weaker coupling strength) would once again be fulfilled when the applied field is reduced to zero.

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Control and Detection of Singlet-Triplet Mixing in a Random Nuclear Field

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We observed mixing between two-electron singlet and triplet states in a double quantum dot, caused by interactions with nuclear spins in the host semiconductor. This mixing was suppressed when we applied a small magnetic field or increased the interdot tunnel coupling and thereby the singlet-triplet splitting. Electron transport involving transitions between triplets and singlets in turn polarized the nuclei, resulting in marked bistabilities. We extract from the fluctuating nuclear field a limitation on the time-averaged spin coherence time T_2^* of 25 nanoseconds. Control of the electron-nuclear interaction will therefore be crucial for the coherent manipulation of individual electron spins.

A single electron confined in a GaAs quantum dot is often referred to as artificial hydrogen. One important difference between natural and artificial hydrogen, however, is that in the first, the hyperfine interaction couples the electron to a single nucleus, whereas in artificial hydrogen, the electron is coupled to about one million Ga and As nuclei. This creates a subtle interplay between electron spin eigenstates affected by the ensemble of nuclear spins (the Overhauser shift), nuclear spin states affected by time-averaged electron polarization (the Knight shift), and the flip-flop mechanism that trades electron and nuclear spins (*1*, *2*).

The electron-nuclear interaction has important consequences for quantum information processing with confined electron spins (*3*). Any randomness in the Overhauser shift introduces errors in a qubit state, if no correcting measures are taken (*4*–*6*). Even worse, multiple qubit states, like the entangled states of two coupled electron spins, are redefined by different Overhauser fields. Characterization and control of this mechanism will be critical both for identifying the problems and finding potential solutions.

We studied the implications of the hyperfine interaction on entangled spin states in two coupled quantum dots—an artificial hydrogen molecule—in which the molecular states could be controlled electrically. A random polarization of nuclear spins creates an inhomogeneous effective field that couples molecular singlet and triplet states and leads to new eigenstates that are admixtures of these two. We used transport measurements to determine the degree of mixing over a wide range of tunnel coupling and observed a subtle dependence of this mixing on magnetic field. We found that we could controllably suppress the mixing by increasing the singlet-triplet splitting. This ability is crucial for reliable two-qubit operations such as the SWAP gate, which interchanges the spin states of the two dots (*3*).

Furthermore, we found that electron transport itself acts back on the nuclear spins through the hyperfine interaction, and time-domain measurements revealed complex, often bistable, behavior of the nuclear polarization. Understanding the current-induced nuclear polarization is an important step toward electrical control of nuclear spins. Such control will be critical for electrical generation and detection of entangled nuclear spin states (*7*) and for transfer of quantum information between electron and nuclear spin systems (*8*, *9*). It may also be possible to control the nuclear field fluctuations themselves in order to achieve longer electron spin coherence times (*10*–*12*).

We investigated the coupled electron-nuclear system using electrical transport measurements through two dots in series (*13*), in a regime where the Pauli exclusion principle blocks current flow (*14*, *15*). The dots were defined with electrostatic gates on a GaAs/AlGaAs heterostructure (Fig. 1E) (*16*). The gate voltages were tuned such that one electron always resides in the right dot, and a second electron could tunnel from the left reservoir, through the left and right dots, to the right reservoir (Fig. 1D). This current-carrying cycle can be described with the occupations (m, n) of the left and right dots: $(0,1) \rightarrow (1,1) \rightarrow$

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(0,2) → (0,1). When an electron enters from the left dot, the two-electron system forms either a molecular singlet, S(1,1), or a molecular triplet, T(1,1). From S(1,1), the electron in the left dot can move to the right dot to form S(0,2). From T(1,1), however, the transition to (0,2) is forbidden by spin conservation [T(0,2) is much higher in energy than S(0,2)]. Thus, as soon as T(1,1) is occupied, further current flow is blocked (we refer to this effect as Pauli blockade).

A characteristic measurement of this blockade is shown in Fig. 1A. The suppression of current (<80 fA) in the region defined by dashed lines is a signature of Pauli blockade (*14*, *15*) (fig. S1 and supporting text). Fig. 1B shows a similar measurement, but with a much weaker interdot tunnel coupling *t*. Strikingly, a large leakage current appears in the Pauli blockaded region, even though the barrier between the two dots is more opaque. Furthermore, this leakage current was substantially reduced by an external magnetic field of only 100 mT (Fig. 1C). Such a strong field dependence is unexpected at first glance, because the in-plane magnetic field, B_{ext}, couples primarily to spin but the Zeeman energies (E_Z) involved are very small ($E_Z \sim 2.5$ μeV at $B_{ext} = 100$ mT, as compared with a thermal energy of ~15 μeV at 150 mK, for example).

Leakage in the Pauli blockade regime occurs when singlet and triplet states are coupled. The T(1,1) that would block current can then transition to the S(1,1) state and the blockade is lifted (Fig. 1D). As we will show, coupling of singlets and triplets (Fig. 1, B and C) in our measurements is caused by the hyperfine interaction between the electron spins and the Ga and As nuclear spins [other leakage mechanisms can be ruled out (supporting text)].

The hyperfine interaction between an electron with spin $\vec{S}$ and a nucleus with spin $\vec{I}$ has the form $(A\vec{I} \cdot \vec{S})$, where A characterizes the coupling strength. An electron coupled to an ensemble of n nuclear spins experiences an effective magnetic field $\vec{B_N} \sim \frac{1}{(g\mu_B)} \sum_i^n A_i \vec{I}_i$, with g the electron g factor and μ_B the Bohr magneton (*1*). For fully polarized nuclear spins in GaAs, $B_N \sim 5$ T (*17*). For unpolarized nuclear spins, statistical fluctuations give rise to an effective field pointing in a random direction with an average magnitude of 5 T/$\sqrt{n}$ (*4*, *5*, *18*). Quantum dots like those measured here contain $n \sim 10^6$ nuclei, so $\|\vec{B_N}\| \sim 5$ mT.

Nuclei in two different dots give rise to effective nuclear fields, $\vec{B_{N1}}$ and $\vec{B_{N2}}$, that are uncorrelated. Although the difference in field $\Delta\vec{B_N} = \vec{B_{N1}} - \vec{B_{N2}}$ is small, corresponding to an energy $E_N \equiv g\mu_B\|\Delta\vec{B_N}\| \sim 0.1$ μeV, it nevertheless plays a critical role in Pauli blockade. The (1,1) triplet state that blocks current flow consists of one electron on each of the two dots. When these two electrons are subject to different fields, the triplet is mixed with the singlet and Pauli blockade is lifted. For instance, an inhomogeneous field along $\hat{z}$ causes the triplet $|T_0\rangle = \frac{1}{\sqrt{2}}(|\uparrow\downarrow\rangle + |\downarrow\uparrow\rangle)$ to evolve into the singlet $\frac{1}{\sqrt{2}}(|\uparrow\downarrow\rangle - |\downarrow\uparrow\rangle)$. Similarly, the evolution of the other two triplet states, $|T_+\rangle = |\uparrow\uparrow\rangle$ and $|T_-\rangle = |\downarrow\downarrow\rangle$, into the singlet is caused by $\hat{x}$ and $\hat{y}$ components of $\Delta\vec{B_N}$.

The degree of mixing by the inhomogeneous field depends on the singlet-triplet energy splitting, E_{ST}. Singlet and triplet states that are close together in energy ($E_{ST} \ll E_N$) are strongly mixed, whereas the perturbation caused by the nuclei on states far apart in energy ($E_{ST} \gg E_N$) is small.

The singlet-triplet splitting depends on the interdot tunnel coupling *t* and on the detuning of left and right dot potentials Δ_{LR}. Δ_{LR} and *t* were controlled experimentally with gate voltages (Fig. 1E). Gate voltage V_t controlled the interdot tunnel coupling. V_L and V_R set the detuning, and thereby determined whether transport was inelastic (detuned levels), resonant (aligned levels), or blocked by Coulomb blockade (Fig. 1F). The coupling of the dots to the leads was held constant with V_{lead}.

The effect of the two tunable parameters *t* and Δ_{LR} on the singlet and triplet energies is illustrated in Fig. 2, A and B. For weak tunnel coupling ($t \sim 0$), and in the absence of a hyperfine interaction ($E_N \sim 0$), the (1,1) singlet and (1,1) triplet states are nearly degenerate (Fig. 2A). A finite interdot tunnel coupling *t* leads to an anticrossing of S(1,1) and S(0,2). The level repulsion results in an increased singlet-triplet splitting that is strongly dependent on detuning (Fig. 2B). At the resonant condition ($\Delta_{LR} = 0$, aligned levels), the

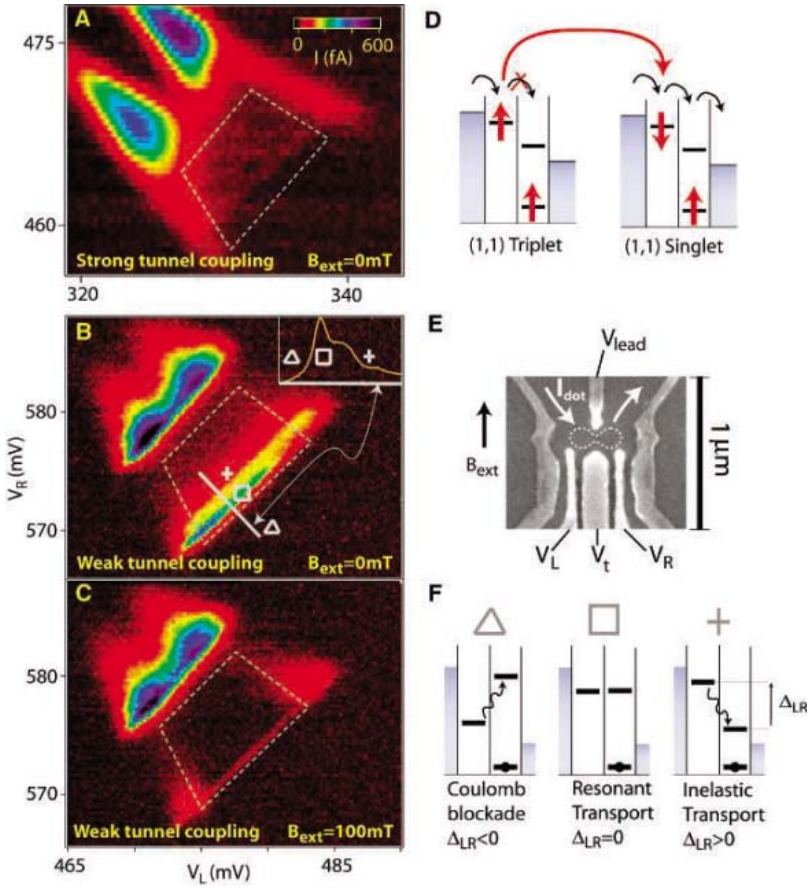

Fig. 1. Pauli blockade and leakage current. (**A**) Color-scale plot of the current through two coupled dots as a function of the left and right dot potentials (voltage bias, 800 μeV; $V_t = -108$ mV). The experimental signature of Pauli blockade is low current (<80 fA) in the area denoted by dashed gray lines. (**B**) Analogous data for smaller interdot tunnel coupling ($V_t = -181$ mV), with the same color scale as in (A). A marked increase of leakage current is seen in the lower part of the Pauli blockaded area (the green and yellow band). Inset: One-dimensional trace along the solid gray line, with Coulomb blockaded, resonant, and inelastic transport regimes marked as defined in (F). (**C**) Analogous data for the same tunnel coupling as in (B), but for $B_{ext} = 100$ mT. The leakage current from (B) is strongly suppressed. (**D**) Two level diagrams that illustrate Pauli blockade in coupled quantum dots. When the (1,1) triplet evolves to a (1,1) singlet (red arrow), Pauli blockade is lifted. (**E**) Scanning electron micrograph showing the device geometry. White arrows indicate current flow through the two coupled dots (dotted line). (**F**) Level diagrams illustrating three transport regimes. △: Coulomb blockade; transport would require absorption of energy. □: Resonant transport; the dot levels are aligned. +: Inelastic transport; energy must be transferred to the environment, for instance, by emitting a phonon.

two new singlet eigenstates are equidistant from the triplet state, both with $E_{ST} = \sqrt{2}t$. For finite detuning (finite but still smaller than the single dot S-T splitting), one singlet state comes closer to the triplet state ($E_{ST} \sim t^2/\Delta_{LR}$), whereas the other moves away. In Fig. 2, A and B, singlet and triplet states are pure eigenstates (not mixed), and therefore Pauli blockade would be complete.

The additional effect of the inhomogeneous nuclear field is shown in Fig. 2, C and D. For small t ($\sqrt{2}t$, $t^2/\Delta_{LR} < E_N$), the (1,1) singlet and (1,1) triplet are close together in energy and therefore strongly mixed (purple lines) over the entire range of detuning. For t such that $t^2/\Delta_{LR} < E_N < \sqrt{2}t$, triplet and singlet states mix strongly only for finite detuning. This is because E_{ST} is larger than E_N for aligned levels but smaller than E_N at finite detuning. For still larger t ($\sqrt{2}t$, $t^2/\Delta_{LR} > E_N$, not shown in Fig. 2), mixing is weak over the entire range of detuning. In the cases where mixing between S and T is strong, as in Fig. 2, C and D (for large detuning), Pauli blockade is lifted and a leakage current results.

The competition between E_{ST} and E_N can be seen experimentally by comparing one-dimensional traces of leakage current as a function of detuning over a wide range of t (Fig. 3A). Resonant current appears as a peak at $\Delta_{LR} = 0$ and inelastic leakage as the shoulder at $\Delta_{LR} > 0$ (*19*). When the interdot tunnel coupling was small, both resonant and inelastic transport were allowed because of singlet-triplet mixing, and both rose as the middle barrier became more transparent. As the tunnel coupling was raised further, a point was reached where E_{ST} became larger than the nuclear field and Pauli blockade suppressed the current (Fig. 1A). The maximum resonant current occurred at a smaller value of t compared to the maximum inelastic current (Fig. 3A,

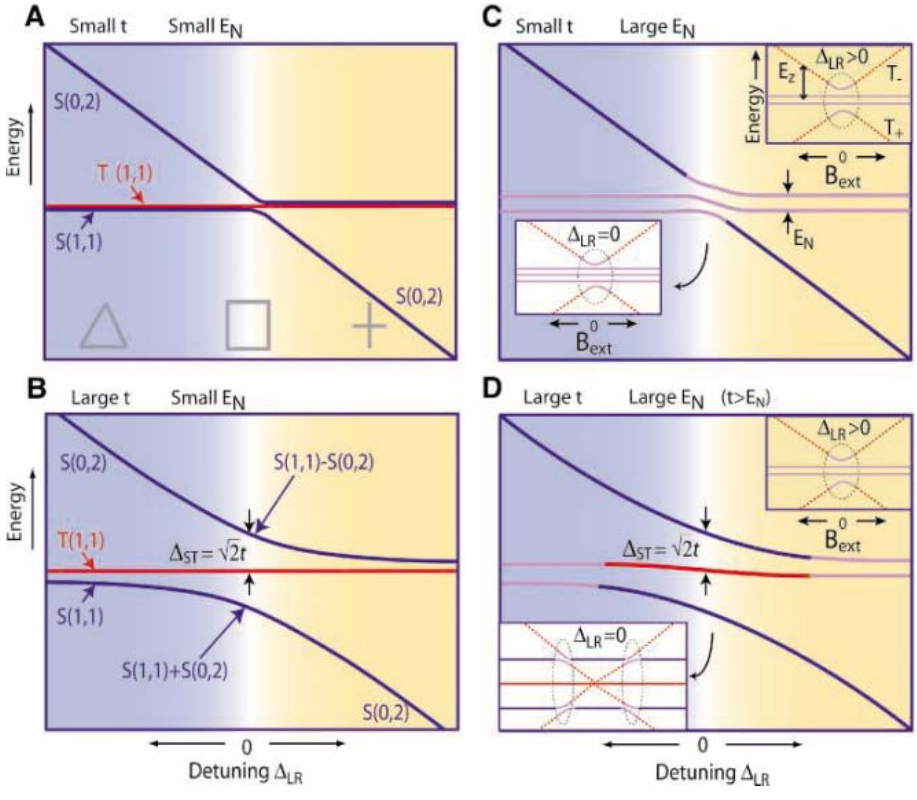

Fig. 2. Two-electron level diagrams showing energy as a function of detuning Δ_{LR}. Detuning is defined so that the energy of T(1,1) remains constant as Δ_{LR} varies (fig. S1B and supporting text). T(0,2) is not shown as it occurs far above the energies shown here. The panels on the left illustrate the effect of t; the panels on the right include the additional effect of an inhomogeneous magnetic field. Pure singlet and triplet states are drawn in blue and red, respectively; strong admixtures are in purple. The blue (△), white (□), and yellow (+) background corresponds to the Coulomb blockade, resonant, and inelastic transport regimes, respectively. (**A**) For small tunnel coupling, T(1,1) and S(1,1) are nearly degenerate. (**B**) For finite t, level repulsion between the singlet states results in a larger singlet-triplet splitting than shown in (A), which depends on detuning. The tunnel coupling does not mix singlet and triplet states. For large Δ_{LR} (that are still smaller than the single dot S-T splitting), $E_{ST} \sim t^2/\Delta_{LR}$. (**C** and **D**) An inhomogeneous field mixes triplet and singlet states that are close in energy (purple lines). For clarity, only one triplet state is shown in the main panels. (C) For small t, T(1,1) and S(1,1) mix strongly over the full range of detuning. (D) For large t, T(1,1) mixes strongly with the singlet only for large detuning. The insets to (C) and (D) show the effect of an external magnetic field on the two-electron energy levels. All three triplets are shown in the insets; the triplets $|T_+\rangle$ and $|T_-\rangle$ split off from $|T_0\rangle$ because of B_{ext}. The leakage current is highest in the regions indicated by black dotted ellipses.

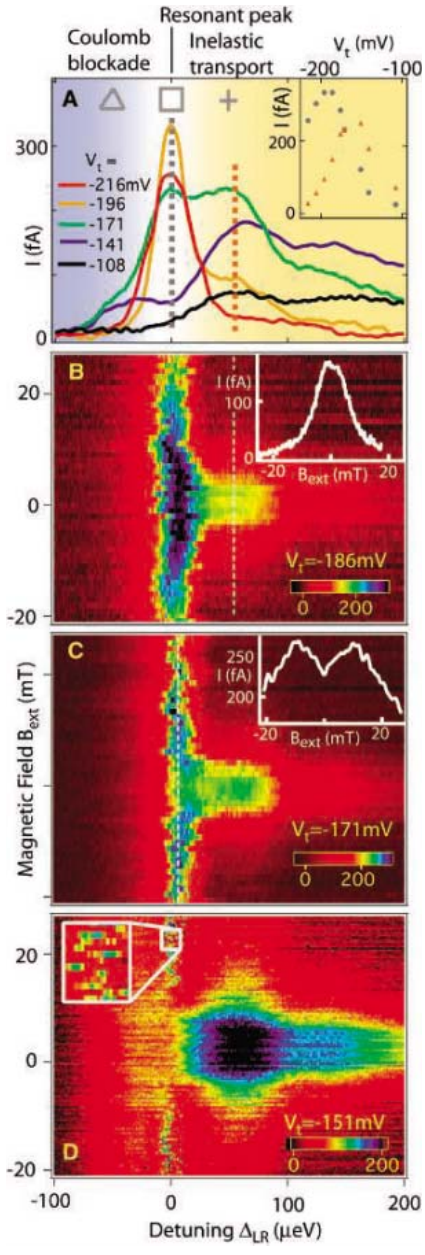

Fig. 3. The measured leakage current results from a competition between E_N, E_{ST}, and E_Z. (**A**) One-dimensional traces of the leakage current as a function of detuning at $B_{ext} = 0$, for a wide range of tunnel couplings (analogous to the inset of Fig. 1B). Coulomb blockade, resonant transport, and inelastic transport are indicated as in Fig. 2. Inset: Leakage current along the dotted gray and orange lines is shown as a function of V_t. Resonant and inelastic leakage (gray and orange markers) reach a maximum at different tunnel couplings ($V_t = -190$ mV and -150 mV, respectively). (**B**) For small tunnel coupling ($<E_N$), both the resonant and inelastic leakage currents drop monotonically with B_{ext}. Inset: Magnetic field dependence of the inelastic current along the dotted line ($\Delta_{LR} = 40$ μeV). (**C**) For larger t ($>E_N$), the resonant leakage current is maximum at $B_{ext} = 10$ mT. Inset: Field dependence of the resonant peak height (dotted line). (**D**) For still larger t, the resonant current is strongly reduced at low field (main panel), then becomes unstable for higher field (inset).

inset). This is consistent with E_{ST} being much smaller for finite detuning than for aligned levels ($t^2/\Delta_{LR} \ll \sqrt{2}t$) (Fig. 2, B and D).

The experimental knob provided by electrostatic gates is very coarse on the energy scales relevant to the hyperfine interaction. However, the external magnetic field can easily be controlled with a precision of 0.1 mT, corresponding to a Zeeman splitting (2 neV) that is 50 times smaller than E_N. For this reason, monitoring the field dependence allowed a more detailed examination of the competing energy scales E_{ST}, E_Z, and E_N.

The competition between E_Z and E_N is clear for small interdot tunnel coupling (Fig. 3B). Leakage current was suppressed monotonically with the magnetic field, on a scale of ~5 mT and ~10 mT for inelastic and resonant transport, respectively. The qualitative features of this field dependence can be understood from the insets to Fig. 2C. At zero field, all states are mixed strongly by the inhomogeneous nuclear field, but when E_Z exceeds E_N, the mixing between the singlet and two of the triplet states ($|T_+\rangle$ and $|T_-\rangle$) is suppressed. An electron loaded into either of these blocks further current flow, explaining the disappearance of leakage at high field in the measurement.

The magnitude of the fluctuating Overhauser field can be extracted from the inelastic peak shape in the limit of small t (Fig. 3B, inset). We fit the data to a model that describes the transport cycle with the density matrix approach (*20*) (supporting text). From this fit, we found the magnitude of the inhomogeneous field $\sqrt{\langle \Delta B_N^2 \rangle} = 1.73 \pm 0.02$ mT ($E_N = 0.04$ μeV), largely independent of Δ_{LR} over the parameter range studied (*21*). The value for the effective nuclear field fluctuations in a single dot was obtained from the relation $\langle B_N^2 \rangle = \frac{1}{2}\langle \Delta B_N^2 \rangle$, giving $\sqrt{\langle B_N^2 \rangle} = 1.22$ mT. This is consistent with the strength of the hyperfine interaction in GaAs and the number of nuclei that are expected in each dot (*4*, *22*).

The three-way interplay between E_{ST}, E_Z, and E_N is most clearly visible in the resonant current. At an intermediate value of tunnel coupling, $t \gtrsim E_N$ (Fig. 3C), the resonant peak was split in magnetic field, with maxima at ±10 mT (Fig. 3C, inset). The lower inset to Fig. 2D illustrates this behavior. At $B_{ext} = 0$, the resonant current in Fig. 3C was suppressed compared to the current in Fig. 3B, because E_{ST} was greater than E_N at that point. Increasing B_{ext} enhanced the mixing as the $|T_+\rangle$ and $|T_-\rangle$ states approached the singlet states. The maximum leakage occurred when the states crossed, at E_{ST} (= $\sqrt{2}t$) = E_Z. Here, E_Z was 0.25 ± 0.03 μeV at the current maximum, from which we extract $t = 0.18 \pm 0.02$ μeV for this setting of V_t. At still larger B_{ext}, $|T_+\rangle$ and $|T_-\rangle$ moved away from the singlet states again, and the leakage current was suppressed.

The system entered into a new regime for still higher tunnel coupling (Figs. 3D and 4), where it became clear that the electron-nuclear system is dynamic. The zero field resonant leakage was further suppressed, and above 10 mT, prominent current spikes appeared (Fig. 3D, inset). The spikes are markedly visible in a three-dimensional surface plot of leakage over a broader range of field (Fig. 4A). For fixed experimental parameters, the current still fluctuated in time (Fig. 4B).

We found that time-dependent behavior was a consistent feature of resonant transport for (E_{ST}, E_Z) ≫ E_N. For some device settings, the time dependence was fast (for example, the fluctuations in Fig. 4, A and B), but for others, the leakage changed much more slowly (Fig. 4C). Starting from an equilibrium situation (bias voltage switched off for 5 min), the current was initially very small after the bias was turned on. It built up and then saturated after a time that ranged from less than a second to several minutes. This time scale depended on Δ_{LR}, t, and B_{ext}. When no voltage bias was applied, the system returned to equilibrium after ~80 s at 200 mT. Similar long time scales of the nuclear spin-lattice relaxation times have been reported before in GaAs systems (*23*) and quantum dots (*24*). We thus associate the slow time dependence observed in our system with current-induced dynamic nuclear polarization and relaxation.

Evidence that the fast fluctuations too are related to current-induced nuclear polarization (and cannot be explained by fluctuating background charges alone) is found in their dependence on sweep direction and sweep rate (*23*, *25*). When the magnetic field was swept while fixed Δ_{LR} was maintained, the current showed fluctuations at low field but suddenly became stable at high field (Fig. 4D). The crossover from unstable to stable behavior occurred at a field that was hysteretic in sweep direction (Fig. 4D), and this hysteresis became more pronounced at higher sweep rates (faster than ~1 mT/s). The connection between the fluctuations and nuclear polarization is also evident from time traces, in which instability developed only after the nuclear polarization was allowed to build for some time (fig. S3).

Unlike the regular oscillations that have been observed in other GaAs structures (*1*, *26*),

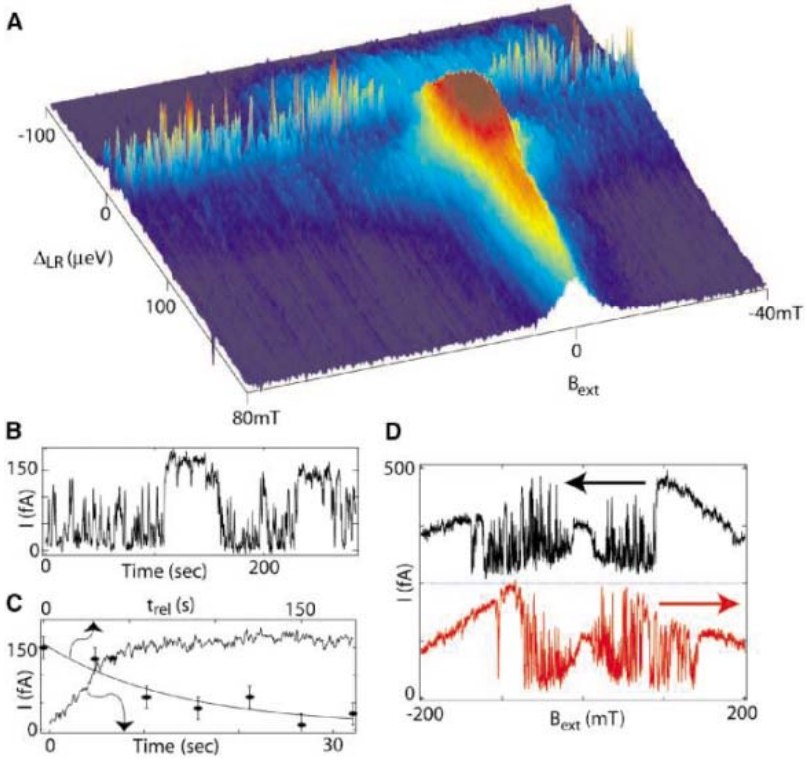

Fig. 4. Time dependence of the leakage current reveals the dynamics of the electron-nuclear system. This time dependence occurs in the regime corresponding to Fig. 2D. (**A**) Surface plot of electrical transport for $V_t = -151$ mV. Instability on the resonant peak is visible as sharp current spikes. The sweep direction is from positive to negative Δ_{LR}, for fields stepped from negative to positive B_{ext}. (**B**) Explicit time dependence of the resonant current exhibits bistability ($V_t = -141$ mV, $B_{ext} = 100$ mT). (**C**) Lower axis: Dynamic nuclear polarization due to electron transport through the device ($V_t = -141$ mV, $\Delta_{LR} = 0$, $B_{ext} = 200$ mT), after initialization to zero polarization by waiting for 5 min with no voltage applied. Top axis: In order to measure the nuclear spin relaxation time, we waited for the current to saturate, switched off the bias voltage for a time t_{rel}, and then remeasured the leakage current. An exponential fit gives a time constant of 80 ± 40 s (measurements of these long time scales result in large error bars, ±20 fA, because of 1/f noise). (**D**) The field dependence of the resonant current is hysteretic in the sweep direction ($V_t = -149$ mV). Each trace takes ~7 min.

the fluctuations in our measurements were random in time and, in many cases, suggested bistability with leakage current moving between two stable values. We discuss the origin of such fast bistable fluctuations in the supporting text.

The ensemble of random nuclear spins that gives rise to the mixing of two-electron states as observed in this experiment also gives rise to an uncertainty of $g\mu_B \sqrt{\langle B_N{}^2 \rangle} = 0.03$ μeV in the Zeeman splitting of one electron. When averaged over a time longer than the correlation time of the nuclear spin bath (~100 μs) (*27*), this implies an upper limit on the time-averaged spin coherence time of $T_2^* = (\frac{\hbar}{2\pi})/g\mu_B\sqrt{\langle \frac{2}{3} B_N{}^2 \rangle} = 25$ ns [as defined by Merkulov *et al.* (*4*)], comparable to the T_2^* found in recent optical spectroscopy measurements (*28*). This value is four orders of magnitude shorter than the theoretical prediction for the electron spin T_2 in the absence of nuclei, which is limited only by spin-orbit interactions (*29*–*31*).

One way to eliminate the uncertainty in Zeeman splitting that leads to effective dephasing is to maintain a well-defined nuclear spin polarization (*12*). Many of the regimes explored in this paper show leakage current that is stable when current-induced polarization is allowed to settle for some time. These may in fact be examples of specific nuclear polarizations that are maintained electrically.

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Gas Adsorption Sites in a Large-Pore Metal-Organic Framework

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The primary adsorption sites for Ar and N_2 within metal-organic framework-5, a cubic structure composed of $Zn_4O(CO_2)_6$ units and phenylene links defining large pores 12 and 15 angstroms in diameter, have been identified by single-crystal x-ray diffraction. Refinement of data collected between 293 and 30 kelvin revealed a total of eight symmetry-independent adsorption sites. Five of these are sites on the zinc oxide unit and the organic link; the remaining three sites form a second layer in the pores. The structural integrity and high symmetry of the framework are retained throughout, with negligible changes resulting from gas adsorption.

Metal-organic frameworks (MOFs) have recently emerged as an important class of porous materials for their amenability to design and the flexibility with which their pores can be functionalized (*1*–*3*). In particular, their extraordinary low density (1.00 to 0.20 g/cm^3) and high surface area (500 to 4500 m^2/g) make them ideal candidates for the storage and separation of gases (N_2, Ar, CO_2, CH_4, and H_2) (*4*–*12*). In this context, identifying the gas adsorption sites in MOFs is critically important to our ability to fine-tune those sites, sterically and electronically, in order to achieve the maximum storage capacity and selectivity.

Precise determination of adsorption sites in large-pore materials remains an ongoing challenge, because the characterization techniques that are commonly applied to this problem, such as inelastic neutron scattering (INS) and diffuse reflectance infrared spectroscopy, do not provide adequate information on the structural details of the adsorption sites (*9*, *13*). The recent powder x-ray diffraction (XRD) studies of gases in MOFs (*14*, *15*) do not elucidate the nature of adsorption sites because the MOFs used have very small pores and lack the possibility of adsorbing gases on multiple sites. For large-pore structures, a more precise technique is required to determine whether adsorption sites lie on the metal oxide or the organic moieties, how many exist, and precisely where they are located in the MOF structure.

We report the detailed single-crystal XRD study of Ar and N_2 adsorbed on the internal surface of a large-pore open-framework mate-

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rial, MOF-5 (*4*). We were able to identify the metrics and geometry of five different primary adsorption sites on the $Zn_4O(CO_2)_6$ and the C_6H_4 units of MOF-5 and uncover edgewise C_6H_4···Ar and C_6H_4···N_2 interactions previously unknown in gas-phase studies of aromatic van der Waals complexes.

Diffraction-quality single crystals of MOF-5 were produced by the solvothermal reaction of zinc nitrate with terephthalic acid in *N*,*N*-diethylformamide (*16*). The material used in the present study was initially evacuated ($<10^{-3}$ Torr) of any gas or volatiles, and its stability was checked by thermal gravimetric analysis, which revealed a large temperature range (100° to 470°C) of stability with no observable weight loss (Fig. 1). The crystal specimens were mounted in capillaries connected to a gas manifold for evacuation before being backfilled with Ar or N_2 and flame-sealed. The amount of gas available for adsorption was estimated from the backfill pressure, crystal size, and the dimensions of the capillaries to be about 5 to 10 gas molecules per formula unit of MOF-5, $Zn_4O(BDC)_3$ (BDC is 1,4-benzenedicarboxylate), which is less than half the established saturation uptake for MOF-5 (*4*).

XRD data were collected by using a single crystal diffractometer equipped with a charge-coupled device camera and an open-flow helium cryostat at temperatures between 293 and 30 K (*16*, *17*). To confirm the completeness of the evacuation procedure, we collected data at 30 K on a single crystal of MOF-5 sealed under vacuum. Refinement of these data confirmed the maintainance of the cubic symmetry of MOF-5 and the absence of guest molecules in the pores (largest electron density peak and hole were 0.444 and –0.238 $e^-/Å^3$, respectively) as shown in the thermal ellipsoid plot of Fig. 2A.

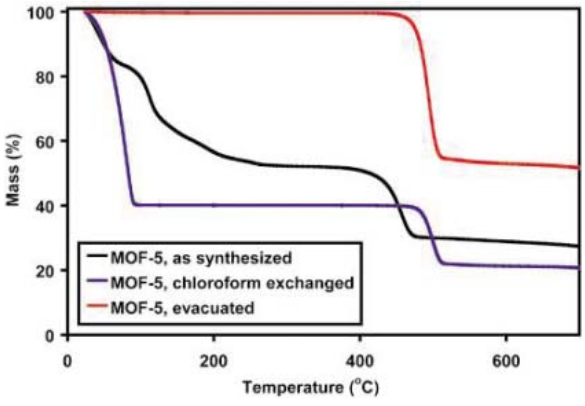

Fig. 1. Thermal behavior of MOF-5 in the as-synthesized, chloroform-exchanged, and evacuated states. The as-synthesized material (black trace) displays a multistep weight loss of 48% up to 300°C, attributed to the desorption of 7.0 *N*,*N*-diethylformamide molecules per formula unit before the onset of decomposition at 410°C. In contrast, the chloroform guests in the exchanged material (blue trace) are completely removed by 100°C (60% weight loss, equivalent to 9.7 chloroform molecules per formula unit). The evacuated framework (red trace) shows no weight loss to 470°C.

Further XRD data were collected from crystals of MOF-5 loaded with Ar or N_2 between 293 and 30 K. Refinement of data collected at 293 K revealed no significant peaks of electron density attributable to guests in the extra-framework region in either case, as would be expected from their weak interaction (Fig. 2, B and C). As the temperature was lowered, many local maxima were located in the observed structure factor (F_{obs}) Fourier maps, providing evidence of the localization of the gas atoms. This localization is most apparent at 30 K in the regions proximal to the $Zn_4O(CO_2)_6$ unit. The shape and amplitude of the electron density contours of Ar and N_2 atoms near this unit are shown in Fig. 2, D and E, respectively. This first site, denoted $\alpha(CO_2)_3$, lies on a triangular face of the octahedron whose vertices are defined by the carboxylate C atoms. This site (Wyckoff site 32*f*, *x* coordinate ~ 0.34) exhibits the highest occupancy in all refinements over the temperature range examined and can be identified as the primary adsorption site. Its symmetry allows the adsorption of up to four adsorbates per formula unit. At this site, the curvature is higher than anywhere else on the framework surface, which allows the adsorbates to interact with three carboxylates and three Zn atoms. The Zn–O and C–O dipoles are presumably most effective in polarizing the gas atoms and lead to comparatively strong interactions.

An additional seven adsorption sites were identified from local maxima of electron density, which are partially occupied by Ar at 30 K. In total, there are five primary adsorption sites, including $\alpha(CO_2)_3$, that are closest to the framework, and three secondary ones that form a second layer of adsorption in the pores (Fig. 3,

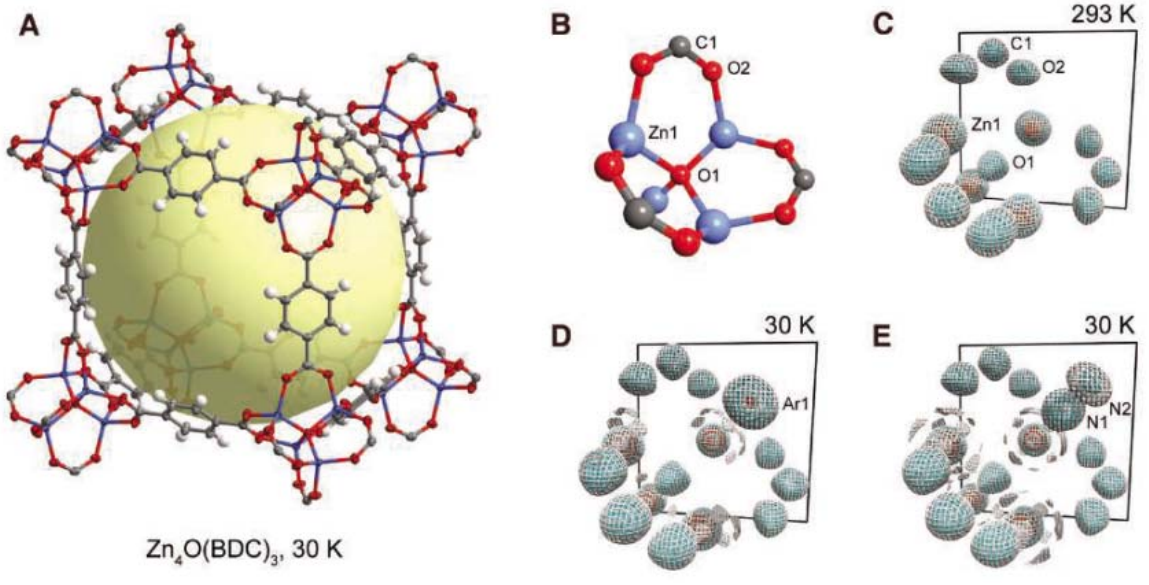

Fig. 2. (**A**) The MOF-5 structure consists of a cubic array of $Zn_4O(CO_2)_6$ units connected by phenylene links (atoms shown as thermal ellipsoids of 90% probability: C, black; H, white; O, red; and Zn, blue), which define large pseudocubic pores that can be completely evacuated. The yellow sphere represents the largest sphere that can occupy the pore without contacting the van der Waals surface of the framework. (**B**) A magnification of the corner of the pore schematically shows the location of adsorption site $\alpha(CO_2)_3$, located equidistant to the three carboxylates. (**C**) The corresponding three-dimensional electron density map (F_{obs}) of this site, determined from single-crystal XRD data obtained at 293 K from crystals loaded under either N_2 or Ar. Only regions of electron density assigned to the framework atoms shown in (**B**) are observed. At 30 K, regions of electron density assigned to adsorbed (**D**) Ar or (**E**) N_2 become prominent. At 30 K, Fourier ripples around the Zn atoms can also be observed. Contours ($e^-/Å^3$): silver, >3; blue, >10; red, >60.

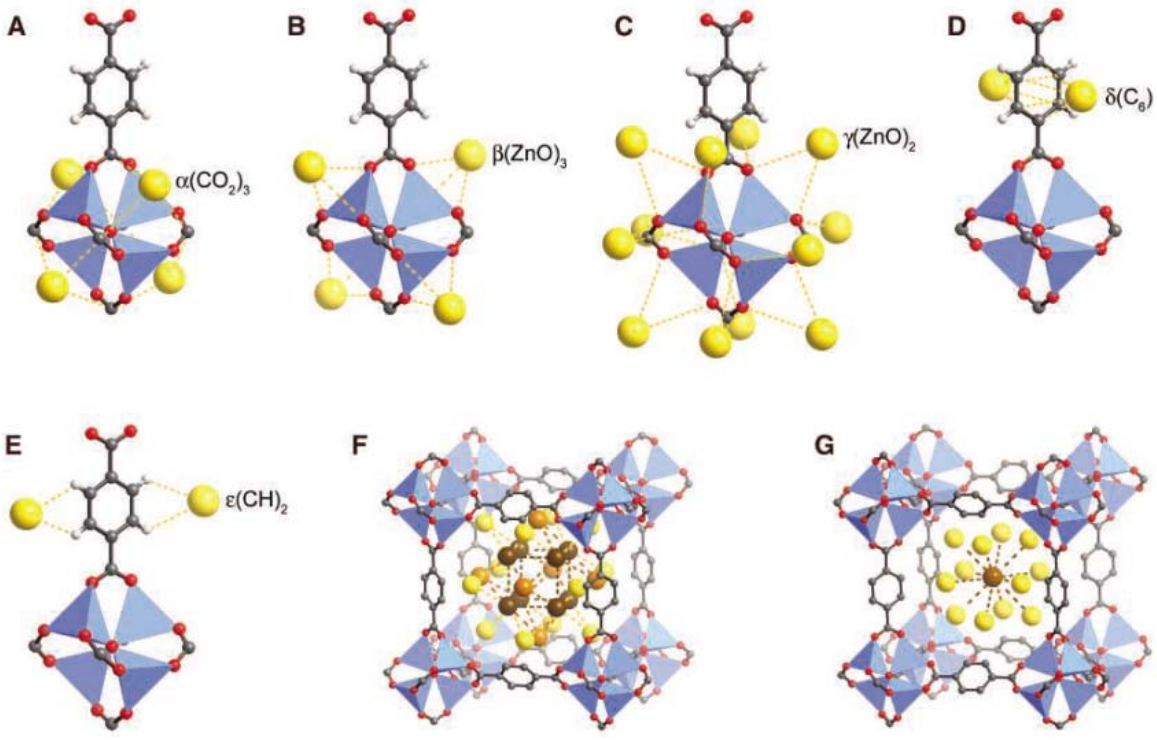

Fig. 3. At 30 K, eight symmetry-independent sites are crystallographically identified as partially occupied by Ar atoms (shown as yellow spheres) in the pores of MOF-5. These include (**A** to **C**) three sites primarily associated with the secondary building unit and those above the (**D**) face and (**E**) edges of the linker. Sites are labeled according to the description in the text. (**F**) Sites ϕ (orange spheres) and η (brown spheres) form a second layer in the large pore above site $\delta(C_6)$ (yellow spheres); (**G**) site θ (brown sphere) is located at the center of the small pore surrounded by site $\varepsilon(CH)_2$ (yellow spheres).

Table 1. Important interatomic distances for Ar adsorbed in MOF-5 at 30 K. Atomic labels correspond to those used in the crystallographic refinements, and primes indicate symmetry equivalents. Dashed entries indicate no close framework contacts.

Adsorption site	Closest framework contacts (Å)		Intersite distances (Å)	
$\alpha(CO_2)_3$	Three Ar(1)···C(1)	3.572	$\alpha(CO_2)_3$···$\gamma(ZnO)_2$	3.677
	Six Ar(1)···O(2)	3.601	$\alpha(CO_2)_3$···η	3.828
	Three Ar(1)···Zn(1)	3.926		
$\beta(ZnO)_3$	Three Ar(2)···O(2)	3.492	$\beta(ZnO)_3$···$\varepsilon(CH)_2$	3.270
	Three Ar(2)···H(3)	3.281	$\beta(ZnO)_3$···$\gamma(ZnO)_2$	3.471
	One Ar(2)···Zn(1)	3.686		
$\gamma(ZnO)_2$	Two Ar(3)···O(2)	3.792	$\gamma(ZnO)_2$···$\beta(ZnO)_3$	3.471
	Two Ar(3)···H(3)	3.306	$\gamma(ZnO)_2$···$\alpha(CO_2)_3$	3.677
			$\gamma(ZnO)_2$···ϕ	3.729
			$\gamma(ZnO)_2$···$\delta(C_6)$	3.820
			$\gamma(ZnO)_2$···$\varepsilon(CH)_2$	3.968
$\delta(C_6)$	Three Ar(4)···C(2)	3.635	$\delta(C_6)$···η	3.652
	Three Ar(4)···C(3)	3.639	$\delta(C_6)$···$\gamma(ZnO)_2$	3.820
			$\delta(C_6)$···ϕ	4.112
$\varepsilon(CH)_2$	Two Ar(5)···H(3)	3.288	$\varepsilon(CH)_2$···$\beta(ZnO)_3$	3.270
			$\varepsilon(CH)_2$···$\varepsilon(CH)_2$	3.770
			$\varepsilon(CH)_2$···$\gamma(ZnO)_2$	3.968
			$\varepsilon(CH)_2$···θ	4.073
ϕ	—		ϕ···η	3.716
			ϕ···$\gamma(ZnO)_2$	3.729
			ϕ···$\delta(C_6)$	4.112
η	—		η···$\delta(C_6)$	3.652
			η···ϕ	3.716
			η···η'	3.763
			η···$\alpha(CO_2)_3$	3.828
θ	—		θ···$\varepsilon(CH)_2$	4.073

A to G). Their respective distances from framework atoms and adjacent sites are summarized in Table 1. In addition to $\alpha(CO_2)_3$, the primary sites include the site above the face of a ZnO_4 tetrahedron, $\beta(ZnO)_3$; the site above the edge of a ZnO_4 tetrahedron, $\gamma(ZnO)_2$; the site above the C_6H_4 phenylene face, $\delta(C_6)$; and the site on the phenylene edge, $\varepsilon(CH)_2$. Two additional sites were observed in the large pore, ϕ and η, as well as a third secondary site at the center of the pore, θ.

The known difficulties associated with the high correlation of site occupancy and thermal displacement parameters were carefully considered, and comparison of the peak heights at these positions with those of the framework atoms in the F_{obs} Fourier maps allowed atomic assignments and subsequent convergence of the least squares refinement with acceptable statistics (*16*). Unlike $\alpha(CO_2)_3$, the remaining seven adsorption sites exhibit smaller relative occupancies, indicating that the binding energies at these sites are similar and less than those at $\alpha(CO_2)_3$. The small occupancies (10 to 20%) of some of these sites reflects the limiting amount of Ar in the capillary; however, it must be noted also that any two sites separated by less than the sum of the van der Waals radii cannot be simultaneously occupied in any one unit cell. For Ar, the nearest neighbor separation of 3.76 Å is taken from the structure of its solid phase (*18*). We note that Ar packing at all adsorption sites (Fig. 3) is not appropriate for all unit cells. At full capacity (28.8 Ar per formula unit) (*4*), it would be necessary to invoke a highly disordered model. One packing arrangement might involve full occupation of sites $\alpha(CO_2)_3$, $\delta(C_6)$, $\varepsilon(CH)_2$, η, and θ and 70% filling of site $\gamma(ZnO)_2$ (corresponding to 28.9 Ar per formula unit), but this model also requires some positional disorder from the high symmetry sites.

Evidence that these sites are intrinsic to MOF-5 was provided by the adsorption of N_2 and our ability to readily observe the adsorption sites from the XRD data, which were determined to be the same as those found for Ar. Even at a temperature as high as 120 K, significant electron density was located at the primary adsorption site $\alpha(CO_2)_3$. At 30 K, the two distinct atoms of the N_2 molecule can be

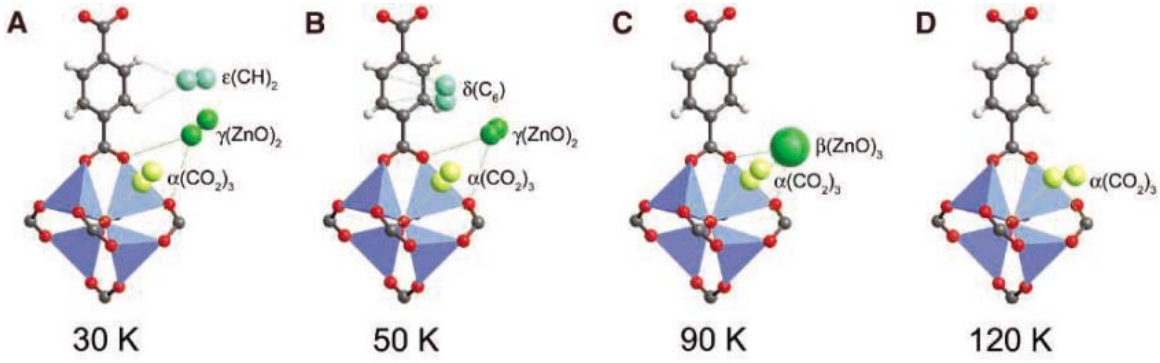

Fig. 4. (**A**) At 30 K, three independent sites are partially occupied by N_2 molecules in the pores of MOF-5, which correspond to sites $\alpha(CO_2)_3$, $\gamma(ZnO)_2$, and $\varepsilon(CH)_2$ of the Ar-loaded structure. Relevant interatomic distances from closest N atom to framework atoms (in Å) include: site $\alpha(CO_2)_3$, 3 C at 3.379, 6 O at 3.372, 3 Zn at 3.606; $\gamma(ZnO)_2$, 2 O at 3.668, 2 H at 3.244; $\varepsilon(CH)_2$, 2 H at 3.097. Intersite distances (measured between molecular centers of mass): $\alpha(CO_2)_3$-$\gamma(ZnO)_2$, 4.104; $\gamma(ZnO)_2$-$\varepsilon(CH)_2$, 4.026; and $\varepsilon(CH)_2$-$\varepsilon(CH)_2'$, 3.708. (**B**) As the temperature is increased, the relative occupancy of these sites changes such that at 50 K site $\delta(C_6)$ is occupied instead of $\varepsilon(CH)_2$; (**C**) at 90 K, site $\beta(ZnO)_3$ is occupied instead of $\gamma(ZnO)_2$, $\delta(C_6)$, or $\varepsilon(CH)_2$; (**D**) at 120 K, only site $\alpha(CO_2)_3$ was occupied. Where refinement was possible, N_2 molecules are shown as their individual atoms, but at 90 K N_2 molecules on site $\beta(ZnO)_3$ appear to be rotationally disordered, and at 120 K there are three possible orientations for N_2 on site $\alpha(CO_2)_3$ (only one is shown).

modeled at three adsorption sites equivalent to $\alpha(CO_2)_3$, $\gamma(ZnO)_2$, and $\varepsilon(CH)_2$ of the Ar-loaded structure (Fig. 4A). For site $\alpha(CO_2)_3$, the molecule is aligned with the threefold axis, allowing a closer approach of one atom (N1) to the framework. Site $\gamma(ZnO)_2$ is also strongly associated with the inorganic cluster, interacting with two separate carboxylate O atoms and two H atoms of the phenylene links. There are 12 of these sites per formula unit (Wyckoff site 96*k*). Site $\varepsilon(CH)_2$ is located at the edges of the phenylene links, and there are six of these per formula unit (Wyckoff site 48*h*). The nearest neighbor (center of mass) distances between sites $\alpha(CO_2)_3$, $\gamma(ZnO)_2$, and $\varepsilon(CH)_2$ are similar to those found in the solid N_2 phases [3.79 to 4.05 Å (*19*)], the dense quadrupolar-ordered phase of N_2 on graphite [4.04 Å (*20*)], and the high-order commensurate structure of N_2 on MgO(100) [3.58 Å (*21*)].

As the temperature is increased to 50 K, site $\delta(C_6)$ is occupied but site $\varepsilon(CH)_2$ is not (Fig. 4B). At 90 K, sites $\gamma(ZnO)_2$, $\delta(C_6)$, and $\varepsilon(CH)_2$ do not show appreciable occupancy; instead, it is primarily at sites $\alpha(CO_2)_3$ and $\beta(ZnO)_3$ (Fig. 4C). At 120 K, only site $\alpha(CO_2)_3$ is partially occupied (Fig. 4D). These results imply a qualitative relation in terms of energy of adsorption for the sites in the order $\alpha(CO_2)_3 \gg \beta(ZnO)_3 > \gamma(ZnO)_2 > \delta(C_6) \sim \varepsilon(CH)_2$. Packing effects cannot be ignored; the separation of the $\beta(ZnO)_3$ and $\gamma(ZnO)_2$ sites is too small for them to be simultaneously occupied, and because $\gamma(ZnO)_2$ has a greater multiplicity (i.e., greater capacity per formula unit), it may become the more important site of the two at lower temperatures to accommodate further adsorbate. The lack of observable electron density at sites ϕ, η, and θ may be attributed to the lesser tendency for N_2 to create an ordered second layer at this small loading.

Our ability to observe and determine the sequential adsorption of gases at well-defined and multiple sites within the large pores of MOF-5 contrasts with other studies performed on molecular materials with much smaller cavities or constricted channels (*14*, *15*, *22*–*24*). In such cases, gases are confined by the narrowness of the pores, and the systems lack the potential for fine-tuning the steric and electronic attributes of the adsorption sites. The openness of MOF-5, however, gives adsorption sites that exhibit partial occupancies through factors related to guests reversibly adsorbing and desorbing and migrating between adjacent sites and the inability to occupy simultaneously sites that are separated by less than the sum of the guests' van der Waals radii. It is not possible to distinguish between these cases with XRD data because they correspond to a time-averaged view of the gas behavior. However, related experimental and theoretical work on small guest molecules in zeolites suggest that each of these situations do occur as a function of temperature (*25*–*28*).

Nevertheless, the presence of multiple adsorption sites in MOF-5 and our ability to precisely locate their positions leads to a rare insight into MOF structures. Specifically, the C_6 phenylene rings are profoundly altered by the presence of the zinc oxide units as evidenced by the observation of Ar and N_2 adsorption sites on the edges of the phenylene ring, $\varepsilon(CH)_2$; a mode, unlike that of the phenylene face, $\delta(C_6)$, previously unobserved in the gas-phase studies of van der Waals complexes of gases with benzene (*29*, *30*). As the MOF-5 structure shows (Fig. 2A), one finds that each C_6 ring is attached to two six-membered rings of –O–C–O–Zn–O–Zn– composition. These heterorings are coplanar to the C_6 ring and undoubtedly alter its electronic character relative to C_6 rings of free benzene, graphite, and carbon nanotubes. Confinement effects enhancing adsorbate-adsorbate interactions may also contribute to the stabilization of binding at these edge sites.

Our findings here also shed some light on the basis for H_2 binding in MOFs. Analysis of INS data collected from MOF-5 as a function of H_2 loading suggested that the favored binding site was near the inorganic secondary building unit (*9*). Additional molecules would then occupy sites closer to or (lastly) on the organic linkers as the loading is increased. However, single-site occupation was not observed in the INS study even at the lowest loading of four H_2 molecules per formula unit, which is equal to the crystallographic multiplicity of this site. This situation is similar to the case in zeolites and suggests that the corrugation of the potential energy surfaces for binding of these molecules is very small. The present study corroborates the qualitative picture developed from the INS studies and provides the missing details of the adsorption sites (Figs. 2 to 4).

The recorded increase of ~200 $Å^3$ (1%) in the unit cell volumes of the materials upon decreasing the temperature from 293 to 30 K suggests that the framework may exhibit negative thermal expansion (*31*). This possibility is being investigated in greater detail by further multitemperature experiments.

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Ar-loaded and N_2-loaded crystal structures (in CIF format) have been deposited as CCDC 277428-277437. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax, (+44)1223-336-033; or deposit@ccdc.cam.ac.uk].
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High Frictional Anisotropy of Periodic and Aperiodic Directions on a Quasicrystal Surface

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Strong friction anisotropy is found when the twofold surface of an atomically clean aluminum-nickel-cobalt quasicrystal slides against a thiol-passivated titanium-nitride tip. Friction along the aperiodic direction is one-eighth as much as that along the periodic direction. This anisotropy, which is about three times as large as the highest value observed in anisotropic crystalline surfaces, disappears after the surface is oxidized in air. These results reveal a strong connection between interface atomic structure and the mechanisms by which energy is dissipated, which likely include electronic or phononic contributions, or both.

The origin of friction and the energy dissipation mechanisms that underlie it are still being explored in fundamental studies. To this day, simple ideas from the times of Leonardo da Vinci, such as the existence of a strong connection between the geometric corrugation profiles (even at the atomic scale) of two contacting surfaces, are still invoked to explain friction (*1*). The idea is that commensurability leads to intimate interlocking and high friction, whereas incommensurability leads to low friction, because the two materials do not come into registry at any length scale. Some of these ideas have been verified recently by rubbing two surfaces of graphite or mica against each other (*2*, *3*) under conditions where wear and plastic deformation are minimized, so that fundamental dissipation forces can be explored. Friction was found to be largest when the crystallographic orientation of the two identical surfaces coincided. Commensurability, however, is only one aspect of the friction problem and does not apply to most interfaces, because the contacting materials are different and therefore almost always incommensurate. Friction anisotropy between incommensurate surfaces has been observed when at least one of the surfaces is crystalline and anisotropic, i.e., when the periodicity changes in different directions. This anisotropy is typically less than a factor of 2 (*4*), although in the case of some organic monolayers on mica, a factor of 3 was observed (*5*).

A different question is whether the existence of periodicity itself is important in friction. For example, it is in periodic systems that the highest thermal and electrical conductivities are found. To explore this question, one could compare the friction properties of a material prepared in crystalline and amorphous forms. This, however, is very difficult, because the two surfaces of the material are likely to be chemically different, which would change the friction properties. As we discuss below, our quasicrystal surface provides a unique example of a material where the periodicity exists only in one direction.

Quasicrystal intermetallics (*6*, *7*), which have long-range atomic order but no periodicity, are ideal samples for exploring this idea, because in two-dimensional quasicrystals, such as decagonal Al-Ni-Co, certain surface terminations exhibit periodic as well as aperiodic atomic ordering along different directions. Quasicrystal surfaces, which are oxidized under ambient conditions, are already known for their high hardness and low friction (*8*–*12*), although these results were obtained under conditions where plastic deformation occurred during sliding. To investigate fundamental aspects of friction on quasicrystals, we cut an Al-Ni-Co single quasicrystal perpendicular to its 10-fold rotational axis to produce a surface

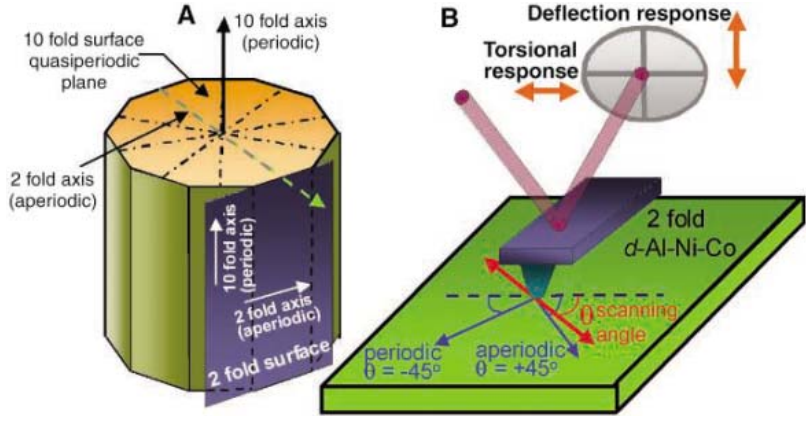

Fig. 1. (**A**) Schematic model of a decagonal Al-Ni-Co quasicrystal, showing the orientation of decagonal and twofold planes. The 2-fold plane is periodic along the 10-fold direction and aperiodic along the 2-fold direction. (**B**) Schematic of the cantilever and the scanning geometry during friction studies.

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with one periodic and one aperiodic axis separated by 90°. The clean, oxide-free surface was prepared and studied under ultrahigh-vacuum (UHV) conditions (*13*).

We used a combined atomic force–scanning tunneling microscope (AFM-STM) to probe the atomic structure of the surface and to perform tribological measurements (*14*). When a clean metal surface and a clean tip come into contact, the adhesion force may be very strong, and surface damage can be unavoidable. This is the case for the TiN/quasicrystal contact (*15*, *16*). Therefore, we passivated the TiN tip with a molecular layer of hexadecane thiol, which substantially reduced the adhesion force. This treatment, coupled with low loads, yielded stable and reproducible contacts that obeyed the Derjaguin-Müller-Toporov (DMT) classical elastic model without hysteresis (*17*). Absence of wear was also confirmed through repetitive imaging.

Figure 1A shows the high-symmetry axes of the quasicrystal superimposed on the typical growth habit. Earlier studies of decagonal Al-Ni-Co (*18*, *19*) indicated that the structure consists of two types of atomic layers stacked in a periodic sequence along the 10-fold direction, with a spacing of 0.2 nm. Each layer has pentagonal (quasicrystalline) symmetry. Thus, the periodicity along the 10-fold direction is 0.4 nm. The twofold plane contains both periodic and aperiodic axes (Fig. 1A).

STM images, such as the one in Fig. 2, confirmed the periodic and aperiodic nature of this surface. On a larger scale, the surface exhibited a terrace-step structure, and the terraces contained rows of protrusions. The protrusions inside the rows were periodically spaced by 0.4 nm, which is consistent with a bulk termination model. The rows, however, are not periodically spaced. The rows with highest contrast, marked by lines, are separated by 0.8 and 1.3 nm. The rows are arranged such that their spacings form a Fibonacci sequence, which is often observed in quasicrystalline materials (*6*). Other features of the rows, such as arrangements of less-prominent rows relative to the main ones and their spacing, are also related to the Fibonacci sequence, which confirms quasicrystallinity in the direction perpendicular to the rows.

The torsional response of the AFM cantilever is proportional to the component of in-plane frictional force perpendicular to the lever axis, whereas the deflection response depends on both the tip-sample force perpendicular to the surface (normal load) and the component of in-plane (frictional) force parallel to the projected lever axis. The angle θ defines the scanning direction relative to the axis of the cantilever (Fig. 1B). In a typical AFM friction experiment, a scanning angle of zero is chosen, so that frictional and normal forces are decoupled. In our experiments, the sample was deliberately oriented such that the 10-fold and 2-fold axes were at $\theta = \pm 45°$, and the lever torsional response was measured as a function of normal load and scanning angle. Because the mechanical response of the AFM lever should be identical for the two directions, an observed asymmetry in torsional response reflects a tip-sample frictional anisotropy. The 2-fold Al-Ni-Co decagonal quasicrystal surface showed high torsional response along the 10-fold (periodic) direction, and low torsional response along the 2-fold (aperiodic) direction (Fig. 3A). The ratio was 7.8 ± 1.3, based on data at $\theta = -45°$ and $+45°$, measured in five different sets of experiments with independent sample and tip preparations. The measured torsional response remained anisotropic for all applied loads (Fig. 3B) throughout the wearless regime. The tip-sample electrical conductance was measured simultaneously and found to be independent of scanning angle, which indicates that the contact area did not change with direction (*20*). The friction curves can be fit well with the DMT elastic model (*17*), which contains only one adjustable parameter. The ratio of the parameters for each curve gives a value of 8.2 ± 0.4 for the friction-force ratio in the periodic and aperiodic directions.

The torsional response of the cantilever is proportional to the product of the frictional force and the cosine of the scanning angle. The component of frictional force along the lever axis causes buckling, which changes the deflection response. If AFM feedback is active, this will cause a change in the normal load, so the measurements of scanning angle–dependent torsional response were carried out with the feedback loop disabled. The sample slope was compensated so that the tip-sample distance, and therefore the applied load, were independent of lateral displacement. Even in open-loop conditions, the buckling component of frictional force modulates the effective normal load, because the tilt of the cantilever relative to the sample surface projects a component of the buckling force normal to the surface. Because the torsional response was measured experimentally by taking the dif-

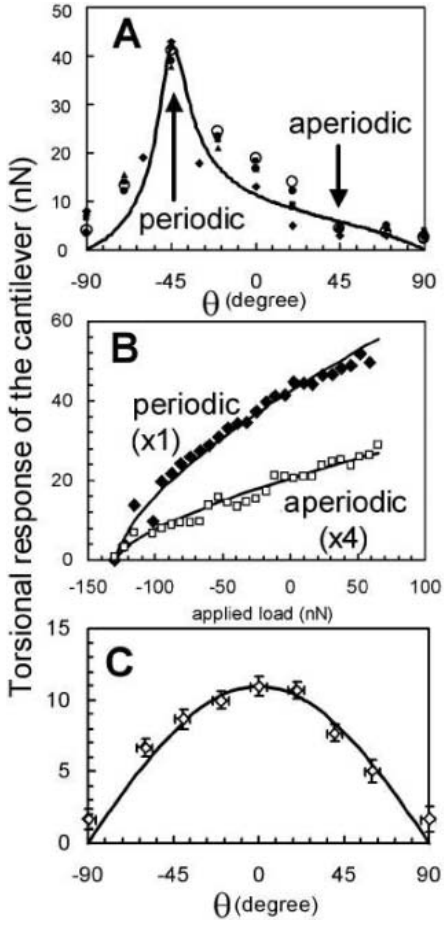

Fig. 3. (**A**) Torsional response of the cantilever measured as a function of scanning angle on the twofold surface of the Al-Ni-Co decagonal quasicrystal. The torsional response was higher along the periodic direction than it was along the aperiodic direction. The solid line shows the calculated torsional response with scanning angle for an anisotropy factor (ratio of friction forces) of 8. The data points are normalized averages of five independent measurements each. (**B**) Torsional response as a function of applied load in both periodic and aperiodic directions. The lines are fits to the DMT model (*17*). The ratio of shear stress in each direction derived from the fits is 8.2 ± 0.4. (**C**) Plot of the torsional response as a function of scanning angle on an isotropic silicon oxide surface with a roughness of <0.3 nm. The solid line shows the calculated torsional response.

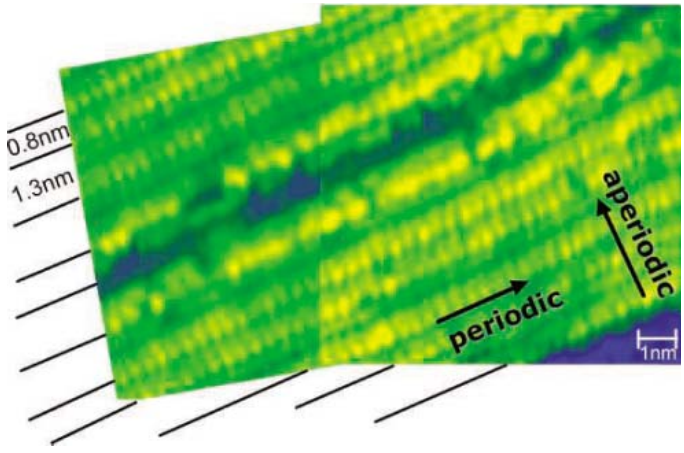

Fig. 2. Collage of two STM images (sample voltage V_s = 1.2 V, current I = 0.1 nA) of the twofold Al-Ni-Co surface. The surface showed a 0.4-nm periodicity along the 10-fold direction. In the direction perpendicular to the atomic rows (twofold direction) a quasiperiodic sequence of 0.8 and 1.3 nm distances was observed.

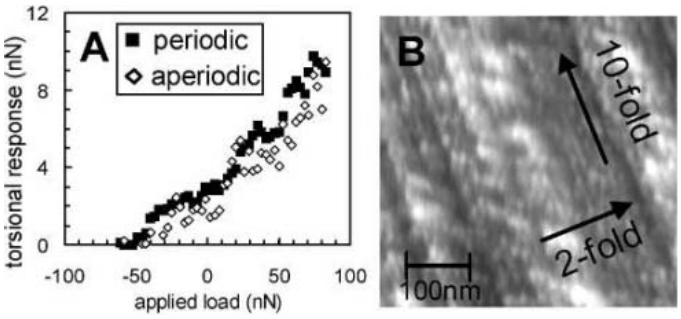

Fig. 4. (**A**) Plot of the torsional response as a function of load along periodic and aperiodic directions after oxidizing the Al-Ni-Co quasicrystal by exposure to air. The anisotropy shown by the clean sample is lost. (**B**) Contact AFM topographical image at an applied load of 0 nN, revealing an amorphous granular oxide film with grain dimensions of 10 to 20 nm. The directions of the atomic rows of the underlying clean substrate are still recognizable.

ference between the signal in the forward and reverse scan directions, the buckling effects cancel out to first order (*21*).

The solid line in Fig. 3A was calculated by assuming an elliptical dependence of friction on scanning direction, with the major axis along the high-friction periodic direction and the minor axis along the low-friction aperiodic direction. The ratio of the major and minor axes corresponds to the friction anisotropy. The magnitude and anisotropy ratio were fit to the experimental torsional response data, giving a reasonable fit for all scanning angles. The experimental procedure was validated by comparing simulation results and torsional response data for an isotropic amorphous silicon-oxide surface, with a root mean square roughness of 0.25 ± 0.06 nm. The squares in Fig. 3C show the measured friction force as a function of θ. It decreases as θ deviates from zero, as expected. The agreement between the experimental data and the simulation (solid line) is excellent.

The friction anisotropy against the thiol-passivated TiN tip disappeared when the surface was oxidized (Fig. 4A) by exposure to air. Extrapolating from studies of icosahedral Al-rich quasicrystals (*22*, *23*), exposure to air at room temperature should form a surface layer of nearly-pure aluminum oxide, 2 to 3 nm thick (Fig. 4B). Hence, the friction anisotropy in Fig. 3B must arise from a short-range interaction between the tip and the surface, which depends on the atomic structure of the clean surface.

Previous macroscopic studies of quasicrystal friction have used two factors to explain the unique tribological properties of these materials: high hardness, which controls the plastic contact area and hence influences friction, and oxide formation (*9*–*12*). Neither factor applies to our study, because oxide-free surfaces were studied under elastic conditions.

Several other factors could account for the observed friction anisotropy. One is an anisotropic response of the hexadecane thiol molecules that coat the tip. The hydrocarbon chains might bend and align parallel to the atomic rows as the tip sweeps along the periodic direction but not when the tip scans perpendicular to the rows. However, the vertical corrugations along periodic and aperiodic directions are only slightly different (0.03 versus 0.04 nm, peak-to-peak) and very small compared with the size of the alkyl chains, which are 0.4 nm in diameter and 2 nm long, so this explanation seems unlikely. Incommensurability between the probe and the surface (*24*) cannot be invoked either, because in our experiments, the TiN tip is amorphous and is covered by alkanethiol molecules, meaning that registry is unlikely in any scan direction.

Two final factors are dissipation by electronic and phononic contributions, where energy is dissipated via excitation and propagation of electron hole pairs and phonons, respectively. These contributions play an important role in bulk electrical and thermal conductivities, which are known to be highly anisotropic in the decagonal phases (*25*, *26*). The transport properties are "normal" along the periodic direction, but anomalous within the aperiodic planes for the decagonal phases. For instance, the bulk thermal conductivity along the periodic direction is larger than it is along the twofold direction by an order of magnitude at room temperature in Al-Ni-Co. The unusual aspects of electron transport in quasicrystals are often associated with electron localization and with the existence of a pseudogap in the electronic density of states at the Fermi level. Phononic friction is also a candidate explanation, because the excitation and propagation of surface phonons along the aperiodic direction could be inhibited by phonon energy gaps (*27*, *28*) predicted theoretically, leading to low energy dissipation. We note that phononic friction is intertwined with the issue of incommensurability, because registry affects the efficiency of phonon excitation (*29*).

It is likely that electronic and/or phononic contributions play an essential role in determining the friction anisotropy caused by the existence of electron and phonon gaps in the aperiodic direction. Our results call for a detailed modeling of the generation and propagation mode of electronic and phonon excitations for this well-defined surface structure.

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Inner Core Differential Motion Confirmed by Earthquake Waveform Doublets

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We analyzed 18 high-quality waveform doublets with time separations of up to 35 years in the South Sandwich Islands region, for which the seismic signals have traversed the inner core as *PKP*(*DF*). The doublets show a consistent temporal change of travel times at up to 58 stations in and near Alaska, and they show a dissimilarity of *PKP*(*DF*) coda. Using waveform doublets avoids artifacts of earthquake mislocations and contamination from small-scale heterogeneities. Our results confirm that Earth's inner core is rotating faster than the mantle and crust at about 0.3° to 0.5° per year.

The Earth's inner core plays an important role in the geodynamo that generates the Earth's magnetic field, and an electromagnetic torque from the geodynamo is expected to drive the inner core to rotate relative to the mantle and crust (*1*–*3*). Song and Richards (*4*) analyzed seismic waves traversing the Earth's fluid and solid cores and reported evidence for a differential inner core rotation. They found that differential travel times between the *BC* and *DF* branches of *PKP* waves (Fig. 1) along the pathway from earthquakes in the South Sandwich Islands (SSI) to a seismic station at College, Alaska (COL), increased systematically by about 0.3 s from 1967 to 1995. The temporal change was interpreted first as a change of the orientation of the fast axis of the inner core anisotropy (*4*), but later and preferably as a shift of lateral velocity gradient in the inner core (*5*, *6*) caused by the inner core rotation. Subsequent studies have provided further support (*5*–*15*), and most estimates of the rotation rate are a few tenths of a degree per year faster than the rotation of the Earth (a super-rotation). However, some studies have failed to resolve a nonzero rotation (*16*, *17*), and claims of a travel-time change have been challenged as artifacts (*18*–*21*).

Waveform doublets can potentially provide much stronger evidence of temporal change by avoiding artifacts of event mislocation and contamination from heterogeneities (Fig. 1, B and C). A waveform doublet is a pair of earthquakes occurring at essentially the same spatial position, as evidenced by their highly similar waveforms at each station recording both events (*22*). Such ideal waveform doublets are commonly found among small earthquakes (*22*, *23*) but are rare for earthquakes large enough to generate *PKP* signals clearly. The high similarity of *BC* and *AB* signals in our doublets is due to propagation paths outside the inner core that sample the same heterogeneities. Observed differences in *DF* give information on changes in the inner core.

Poupinet *et al*. (*20*) developed a method to use pairs of SSI earthquakes to detect inner core rotation, but the earthquake pairs they used were not waveform doublets (*24*). Li and Richards (*12*) reported a waveform doublet in the SSI region (one event in 1987 and the other in 1995), which had clear but weak *DF* signals at the station COL, showing a time shift of 0.10 to 0.15 s in *DF* over 8.5 years. Here, we report observations of 17 additional waveform doublets in the SSI region separated by up to 35 years and detected at up to 58 stations (fig. S1). These doublets show systematic changes in *DF* travel times and coda waveforms, providing strong support for differential inner core rotation.

Waveforms of all the 18 doublets are shown in Fig. 2 and figs. S2 to S6, which are derived from 30 earthquakes that occurred from 1961 to 2004 (tables S1 and S2) (*25*). The *BC* and *AB* waveforms of these event pairs at COL and Beaver Creek array stations (BC01 and BC04) are highly similar (Fig. 2A), with cross-correlation coefficients of 0.79 to 0.99 (table

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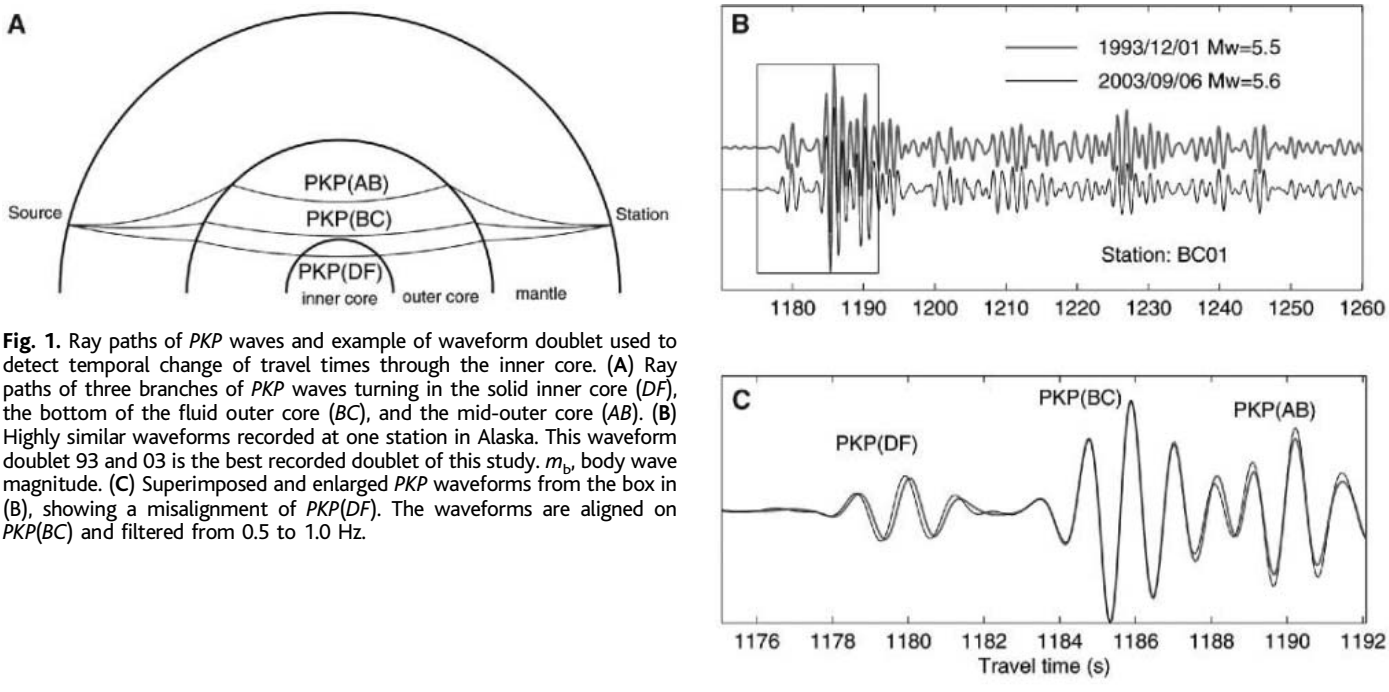

Fig. 1. Ray paths of *PKP* waves and example of waveform doublet used to detect temporal change of travel times through the inner core. (**A**) Ray paths of three branches of *PKP* waves turning in the solid inner core (*DF*), the bottom of the fluid outer core (*BC*), and the mid-outer core (*AB*). (**B**) Highly similar waveforms recorded at one station in Alaska. This waveform doublet 93 and 03 is the best recorded doublet of this study. m_b, body wave magnitude. (**C**) Superimposed and enlarged *PKP* waveforms from the box in (B), showing a misalignment of *PKP*(*DF*). The waveforms are aligned on *PKP*(*BC*) and filtered from 0.5 to 1.0 Hz.

S2). Such waveform similarity allows us to measure relative time shifts with high precision. We measured the relative time shifts of the three phases (i.e., *DF* with *DF*, *BC* with *BC*, and *AB* with *AB*) by time-domain waveform cross correlation. The difference in differential *BC* – *DF* times, $d(BC - DF)$, and the difference in differential *AB* – *BC* times, $d(AB - BC)$, are then derived from the relative shifts of the three phases (table S2). The $d(AB - BC)$ value of each event pair varies from 0.0 to 0.09 s, indicating a difference in epicentral distance of less than 5.5 km (from a standard reference Earth model). Additional stations show similarity of waveforms for 16 of the 18 pairs (fig. S6 and table S2).

When signals from these high-quality waveform doublets are aligned on the *BC* phase, the *DF* phases for event pairs with time separation of less than 4 years overlap with each other well; however, the *DF* phase of the later event arrives consistently earlier than that of the earlier event for doublets separated by more than 4 years, and the *DF* phase is seen to arrive progressively earlier as the time separation increases (Fig. 2B). Also, the waveforms of the *DF* coda become dissimilar when the time separation is larger than 7 to 10 years.

The best recorded example is doublet 93 and 03 [1 December 1993, body wave magnitude (m_b) 5.5; and 6 September 2003, m_b 5.6] (Fig. 1 and figs. S4 and S5). We obtained waveform records for both events at 102 stations distributed over a large range of distances and azimuths. The cross-correlation coefficient is higher than 0.9 for short-period waveforms in a 180-s-long window at most of these stations. A double-difference analysis (*26*) with the use of catalog and hand-picked arrival times and relative travel times from waveform correlation shows the two events are within 1 km horizontally and about 100 m vertically (fig. S7) (*25*). Among the 102 stations, 58 of those in and near Alaska (fig. S1 and table S3) recorded clear *DF* signals. The *DF* amplitudes are particularly strong at Beaver Creek array (fig. S4). The directly observed values of $d(BC - DF)$ are positive for all the 58 stations, which is strong evidence for change in the inner core occurring somewhere along the paths shown in Fig. 3. The doublet was recorded by four arrays at a total of 35 stations (table S3), which can be used to estimate empirically the effect of small mislocation on $d(BC - DF)$ times (fig. S8) (*25*). Our analysis using Eileson array data shows that the effect of plausible mislocation of ~1 km is far too small (about 0.013 s) to cause the observed $d(BC - DF)$ of about 0.1 s (Fig. 3B and table S3). If we use a reference Earth model, the effect is even smaller.

The change in $d(BC - DF)$ between time $T + \Delta T$ and time T can be expressed to first order as $d(BC - DF) = \frac{\Delta\bar{v}}{v_0} t_0$, where $\frac{\Delta\bar{v}}{v_0}$ is the fractional inner core velocity change over the time period ΔT averaged along the ray path in the inner core, and t_0 and v_0 are the travel time and velocity in the inner core for a reference Earth model, respectively. The fractional change in inner core velocity, $\frac{\Delta\bar{v}}{v_0} = \frac{d(BC-DF)}{t_0}$, inferred from the doublets that are separated by more than 4 years is 0.089 ± 0.031% (±SD) over 10 years (Fig. 3B). The temporal change in inner core velocity is about three times the standard deviation and is thus significantly different from zero.

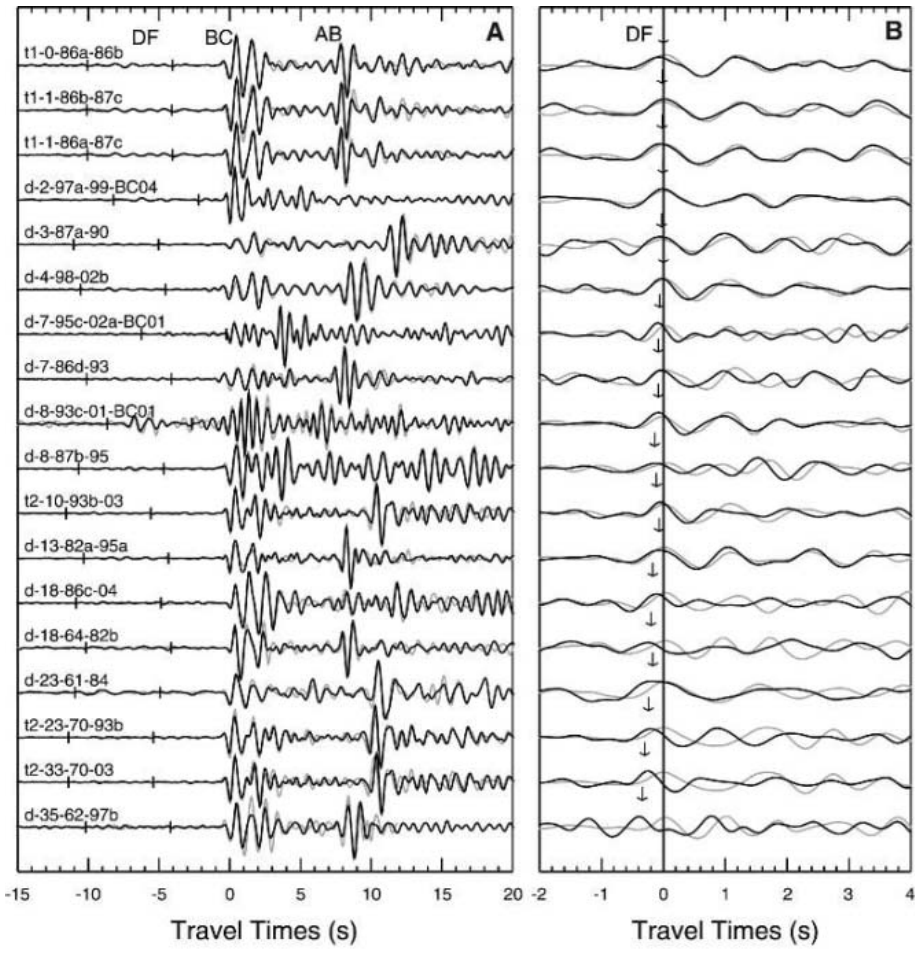

Fig. 2. (**A**) *PKP* waveforms at COL station and Beaver Creek array stations BC01 and BC04 of 17 SSI doublets found in this study and one discovered previously (*12*). The traces (gray for the earlier event, black for the later one) are aligned with the *BC* phase. They are sorted with increasing time separation from top to bottom (the year difference and the years of the two events are indicated in the label). (**B**) Enlarged view of *DF* segments marked by ticks in (A). The traces have been shifted so that the onset of the *DF* arrival of the earlier event of each doublet is roughly aligned. The arrow marks the measured difference of *BC* – *DF* times (table S2).

Among the doublets we discovered (Fig. 2) are two sets of earthquake triplets, which provide another powerful display of systematic travel time change (fig. S3). One set (triplet t1) is separated by less than 2 years; the other set (triplet t2) by 10 to 33 years. Triplet t2 includes the best recorded doublet (93 and 03) and another SSI event in 1970. The similarity of *P* waveforms at stations San Juan, Puerto Rico (SJG) and Scott Base, Antarctica (SBA), which recorded all three events, confirms that the three events indeed occurred at the same location or nearby locations (fig. S3E). The *DF* arrival times agree well with each other in each pair of the triplet t1, but the *DF* of the later event in pairs of triplet t2 is early by 0.11, 0.24, and 0.34 s, respectively, as the time separation increases from 10, to 24, and to 33 years.

Most of our doublets were recorded at COL with clear *DF* phase (Fig. 2). Our discovery of the doublets with large time separation makes it possible to observe large time shifts in the *BC* – *DF* differential time, and the numerous doublets make it possible to characterize the uncertainty of the inferred temporal change. Figure 4 shows consistent increase of our measured $d(BC - DF)$ values at COL with time separation. The data show very small scatter, tightly constraining estimates of the rate of the temporal change (table S4). Assuming each doublet consists of colocated events, our estimate of the temporal change is 0.0092 ± 0.0004 s/year (Fig. 4). Correct-

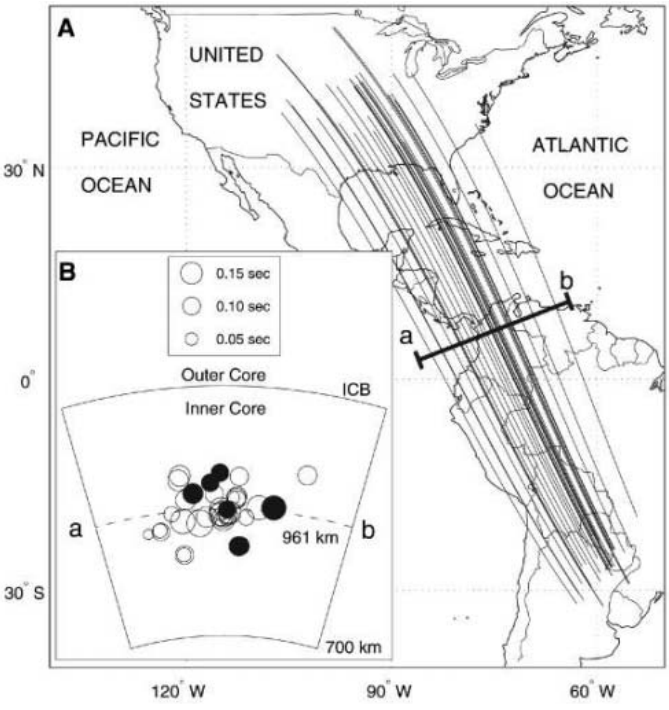

Fig. 3. Paths within the inner core for doublet events separated by more than 4 years, all of which show a positive time shift. (**A**) Map view of *DF* paths projected up to the Earth's surface, including the 93 and 03 doublet, detected by 58 stations; nine additional doublets detected at the College station, Alaska; and two additional doublets detected at the Beaver Creek array. (**B**) Vertical cross-section *a* – *b* showing the *DF* ray turning radius. Open circles are paths to single stations, filled circles are paths to arrays in Alaska, and circle size indicates the measured time change *d*(*BC* – *DF*) for each path, normalized to a 10-year separation. ICB, inner core boundary.

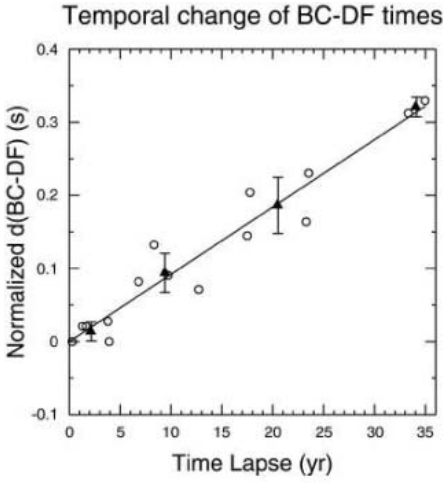

Fig. 4. Difference of *BC* – *DF* times, *d*(*BC* – *DF*), at station COL as a function of the time separation between the two events of the doublet. The error bar indicates the mean (solid triangle) ± SD of the data binned over a 5-year period. The measured *d*(*BC* – *DF*) value has been normalized by the travel time through the inner core and then multiplied by the travel time through the inner core at the average distance of 151°. The line is the linear regression of the data with zero intercept; the slope is 0.0092 s/year with standard deviation 0.0004 s/year.

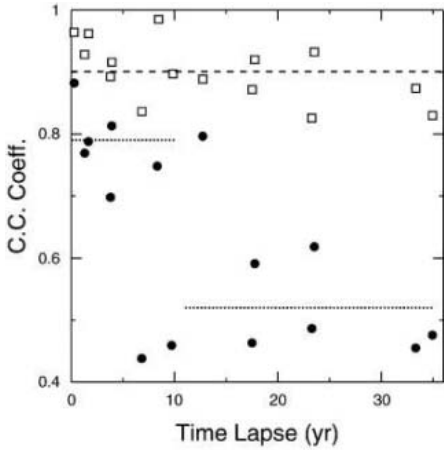

Fig. 5. Cross-correlation coefficients of the *BC* phase (open squares) and the *DF* phase and its coda (solid circles) for the doublets at station COL, as a function of the time separation between the two events of the doublet. The mean of the *BC* values is indicated by the dashed line. The *DF* values seem bimodal and the means of the two groups are indicated by the dotted line segments. The time window used to calculate the *DF* cross-correlation coefficient (C. C. Coeff.) includes the onset of the *DF* phase and its coda up to the onset of the *BC* phase.

ing for event separation of up to a few kilometers, as indicated by the small values of *d*(*AB* – *BC*) times (table S2), changes this result little and gives the estimated temporal change as 0.0090 ± 0.0005 s/year (fig. S9) (*25*). Both these new estimates are about 20 times their measurement errors, which is very strong evidence the travel time is indeed changing. They are also very close to the value first obtained by Song and Richards (*4*), 0.0109 ± 0.0014 s/year. Systematic temporal change is also clear from doublets at Beaver Creek array even though the time separation is much shorter (10 years) (fig. S10).

Our waveform doublets show that the waveforms of the *DF* coda become dissimilar when the time separation is larger than about 7 years (Fig. 2 and fig. S11), providing additional evidence for an inner core motion. Changes in *PKP* coda ascribed to inner core rotation have been noted elsewhere (*15*). Figure 5 shows cross-correlation coefficients of *BC* and *DF* waveforms for each doublet at COL. The *BC* cross correlation fluctuates from 0.82 to 0.98. However, the cross correlation of the *DF* phase and its coda deteriorates sharply from about 0.79 to about 0.52 when the time separation is greater than 7 to 10 years. For doublets with large time separation, the low *DF* cross-correlation coefficients are upper bounds, as cycles may have been skipped in order to find the highest correlation. The *DF* coda is presumably caused by scattering within a complex anisotropic heterogeneous structure (*27*–*29*). So the observed breakdown of waveform similarity is evidence, independent of travel time change, for motion of the inner core.

Assuming the temporal change in *BC* – *DF* times is the result of a shift of the underlying lateral velocity gradient in the inner core due to the rotation around the spin axis (*5*), our new estimated rotation rate is about 0.27° to 0.53° per year (table S5). The biggest uncertainty in determining the inner core rotation rate lies not in estimating the temporal change of travel times but in imaging lateral changes of velocity within the inner core.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/309/5739/1357/DC1
Materials and Methods
Figs. S1 to S11
Tables S1 to S5
References

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Carbon Flux and Growth in Mature Deciduous Forest Trees Exposed to Elevated CO_2

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Whether rising atmospheric carbon dioxide (CO_2) concentrations will cause forests to grow faster and store more carbon is an open question. Using free air CO_2 release in combination with a canopy crane, we found an immediate and sustained enhancement of carbon flux through 35-meter-tall temperate forest trees when exposed to elevated CO_2. However, there was no overall stimulation in stem growth and leaf litter production after 4 years. Photosynthetic capacity was not reduced, leaf chemistry changes were minor, and tree species differed in their responses. Although growing vigorously, these trees did not accrete more biomass carbon in stems in response to elevated CO_2, thus challenging projections of growth responses derived from tests with smaller trees.

How forest trees, the largest biomass carbon (C) pool on Earth, will respond to the continued rise in atmospheric CO_2 is unknown (*1*). Is there a potential for more growth, and perhaps more C storage, as a result of CO_2 fertilization (*2*)? Are trees in natural forests already carbon saturated, given that CO_2 concentrations have already reached twice the glacial minimum concentration (*3*)?

Experimental ecology has made important advances in recent years answering such questions, but unlike grass and shrub vegetation, adult forest trees do not fit any conventional test system with elevated CO_2. Free air CO_2 enrichment (FACE) is currently applied to fast-growing plantations (*4*–*7*), but to date, tall trees in a natural forest have not been studied because of overwhelming technical difficulties. We solved this problem with a technique called web-FACE (*8*) that releases pure CO_2 through a fine web of tubes woven into tree canopies with the help of a construction crane (Fig. 1) (*9*). Here, we present responses of 32- to 35-m-tall trees in a near-natural deciduous forest in Switzerland (*9*) to a 530 parts per million (ppm) CO_2 atmosphere over 4 years. Given the size and species diversity of the study trees, this project inevitably is tree- and not ecosystem-oriented, in contrast to other FACE projects, which use smaller trees. Because plant responses to CO_2 are species-specific (*10*, *11*), insight from single-species approaches remains limited, no matter how large the test scale. The statistical power of our approach is limited at the species level because of reduced intraspecific replication, but tree-ring analysis helps to account for much of the variation in tree vigor. The ultimate effect of rising CO_2 will remain concealed within our limited time scales, yet knowing the dynamics of tree responses over a number of years helps to estimate the non-linear, longer-term trends. The project is thus a compromise between realism and precision, given that there is no way to maximize both (*12*).

Web-FACE uses CO_2 gas with a constant carbon isotope composition ($\delta^{13}C$) of $-29.7 \pm 0.3‰$ (mean ± SE of four annual means). Mixture with ambient-air CO_2 ($\delta^{13}C = -8‰$) results in a ^{13}C tracer signal in photoassimilates that was monitored at ~50 canopy positions with "iso-meters" (small containers planted with a grass using the C_4 photosynthetic pathway), which yielded a 4-year mean of 5.8 ± 0.5‰ ^{13}C tissue depletion. Once assimilated by leaves, the signal penetrates the various biomass compartments and allows one to track the fate of carbon. During the first season, leaves accreted 40% (*Quer-*

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Fig. 1. Canopy CO_2 release by a web of porous tubes (left), with "iso-meters" (front) and gas sampling lines (back) for control, all operated from a 45-m-high crane (right).

cus), 65% (*Fagus*), 67% (*Acer*), 79% (*Carpinus*), and 100% (*Tilia*) of new carbon as compared with the iso-meter mean (*9*). By the end of the first season, new assimilates contributed 71% and 73% to newly formed leaf and wood tissue across all trees (Fig. 2), suggesting very close coupling between leaf and tree-ring construction (*13*). Tree rings of *Quercus* and *Tilia* consisted almost completely of new carbon after the first year, whereas in *Fagus*, the tree-ring signals were weaker and lagged. By the end of the fourth year, 99% of all leaf mass and 91% of all wood mass consisted of new C. After 4 years the new <1-mm fine root fraction (*9*) reached 40% (2.3‰ ^{13}C depletion in 2004), suggesting a ~10-year fine root turnover, similar to the data for other deciduous forests (*14*, *15*).

Soil CO_2 sampled in 170 gas-wells at 3- to 11-cm soil depth across the crane area was 1‰ more depleted after 7 weeks in the treatment zone and 1.8‰ after 4 months, and remained at 2.2 ± 0.2‰ during the remaining test period, indicating a near steady-state efflux of new C (38% of total CO_2 emitted). Soil CO_2 concentrations were 25% higher in the elevated CO_2 area as compared with the control area (P = 0.03) in June 2001 and reached +44% in 2002. Mycorrhizal fungi carried a 64% new carbon signal already by the end of the first year (*16*). CO_2 enrichment thus rapidly produced isotopic fingerprints in the whole leaf-soil continuum. The strong soil-air signal indicates a rapid flux of new carbon through this system, and increased soil CO_2 concentrations provide clear evidence for enhanced metabolic activity in soils under CO_2-enriched trees (*16*), as was found in smaller test systems (*17–19*) and in four forest FACE studies (*4*, *20*).

We found no downward adjustment of photosynthetic capacity in response to growth in elevated CO_2 (*21*), similar to findings for other forest FACE experiments (*22–24*) but contrasting with those for some smaller test systems (*25*). Hence, it is not surprising that leaf nitrogen concentrations in the canopy were little affected (Table 1). Only *Carpinus* showed a significant mean reduction in leaf N by 15%, and across all trees the mean depletion was not significant when the dilution by nonstructural carbohydrates (NSC) was accounted for. *Quercus* and the subdominant taxa showed a significant +22% to +25% (P = 0.005) increase of NSC in leaves in elevated CO_2, similar to that for sweetgum in the Oak Ridge FACE (*23*). Across all trees, NSC increased by 12% (Table 1). NSC concentrations in branch wood were not significantly affected. The slight reductions in N and increases in NSC across trees were largely driven by the presence of two taxa, whereas the 5 to 8% reduction in specific leaf area (SLA) was not species-specific. *Fagus*, the only species with a periodic growth response (see below), did not show a CO_2 response in any of these traits.

Foliage per unit land area (leaf area index, LAI) and leaf duration did not exhibit consistent changes. Elevated CO_2 increased mean leaf duration for 2002 to 2004 in *Carpinus* and *Fagus* by 5 to 6 days and reduced it by 5 days in *Quercus* (marginally significant for the species × CO_2 interaction for *Quercus* and *Fagus*; P = 0.068). Annual litter production did not respond to CO_2 (Fig. 3). It indicates a constant LAI of ~5, in line with data for younger monocultures (*7*, *17–19*, *26*).

Total C and N in fresh fallen litter did not change significantly (*9*), but NSC concentrations (varying from 1.8 to 5.7%) rose, suggesting an overflow of photoassimilates channeled to the decomposer pathway (Table 2). Lignin concentrations ranging from 6.5 to 15% across species in ambient CO_2 were significantly reduced in elevated CO_2, largely driven by *Fagus* and the subdominant taxa. *Quercus* litter was hardly affected. These results indicate a shift in carbon fractions from recalcitrant to more labile compounds. Consequently, litter mass loss was enhanced after 220 days of in situ decay (largely driven by *Carpinus* and the subdominant taxa). In summary, litter became richer in starch and sugar, poorer in lignin, and decomposed faster, but the effect was species-specific. Ecosystem consequences will thus reflect species presence and abundance.

Using pretreatment radial growth rates of trees (tree-ring analysis) (*9*), we standardized basal stem area increments during the treatment period derived from girth tapes. *Fagus* showed a sharp growth stimulation in the first year (+ 92%, P = 0.026), whereas no effects were observed in the other taxa at any time (Fig. 4). The CO_2 response in *Fagus* became insignificant in 2002, but recovered in 2003 (P = 0.028) during a centennial heat wave during which elevated CO_2 improved plant water relations (*27*). The cumulative 4-year response of *Fagus* was only marginally significant at P = 0.07. We performed a second analysis for trees irrespective of species that yielded no significant basal area effect, because the *Fagus* signal was diluted by other species signals and, hence, the cumulative CO_2 signal for all trees in 2004 was zero (P = 0.79), suggesting C saturation (*3*, *28*). Tree-ring chronologies illustrate that the four treatment seasons included a pair of best and worst years over 9 years of growth data, partly explaining the greater variation. Tagged and revisited leading shoots in the top canopy showed no consistent length growth response across species and years (mean annual increment ~15 cm). Fruiting was un-

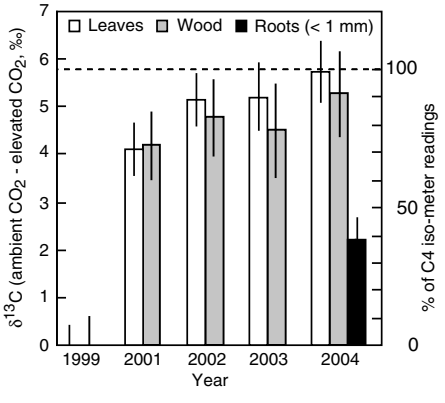

Fig. 2. New carbon in leaves, tree rings, and fine roots as assessed by ^{13}C tracer signals (means ± SE). The maximum steady-state carbon isotope difference of 5.8‰ between ambient and elevated CO_2 is represented by the iso-meter reference value (C_4 grass growing in the canopy, straight dashed line).

Table 1. Leaf nitrogen (N), nonstructural carbohydrates (NSC), and specific leaf area (SLA) across taxa for 13 trees growing in elevated CO_2 versus 16 control trees [repeated measures analysis of variance (ANOVA), with pretreatment values for each individual tree as covariable]; data are for 2001 to 2004, and means for early and late summer are pooled. Dry matter was used as a common reference, either including or excluding NSC.

Leaf trait	Difference (%)	P-value	Main driver
Leaf NSC	+12	0.007	Quercus, TAP*
N, total	−10	0.027	Carpinus, TAP
N, NSC-free	−7	0.136	Carpinus
SLA, total	−8	0.002	Nonspecific
SLA, NSC-free	−5	0.025	Nonspecific

*TAP is a mix of rare taxa of the genera *Tilia*, *Acer*, and *Prunus*.

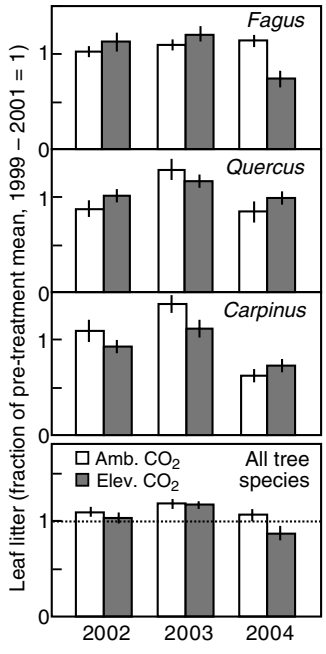

Fig. 3. Leaf litter production standardized (divided) by the mean for two pretreatment years and the year 2001 (*9*) for each litter trap (218 ± 9 g m^{-2} for 56 traps) to account for a priori spatial variability (means ± SE).

affected except for a gain in a single masting year in *Carpinus* (Fig. 5).

The data suggest no lasting growth stimulations by CO_2 enrichment in these mature trees after 4 years. It should be emphasized that the dominant study trees have reached only half of their average life span, have reached about two-thirds of their maximum size, and are growing vigorously by forestry standards (*9*). Other forest FACE experiments with younger trees have either shown a continuous growth stimulation (*Pinus taeda*) (*4*) or a transitory response, very similar to the one shown here for *Fagus* (*Liquidambar styraciflua*) (*5*). A lack of a response in stem growth or leaf litter production does not preclude a faster rate of below-ground (root) production, as was shown for the Oak Ridge FACE (*15*), but these are transitory C pools, which could translate into more recalcitrant forms of carbon in soil humus, mineral nutrients permitting.

Initially we asked whether forest trees will reduce their C uptake or trap more carbon in a CO_2-rich world. Our data suggest that they instead "pump" more carbon through their body. The few secondary effects found varied with species. Had we studied only single species, each would have led us to draw different conclusions: one species reducing its protein concentration, another consistently increasing its leaf carbohydrates, litter being affected in one group of species and not in others, and one species showing a transitory growth stimulation and others not. The Swiss forest FACE study thus points at the crucial role of tree species identity (*29*) and so far does not support expectations of greater carbon binding in tree biomass in such deciduous trees. The lack of a growth response or the transient response in one species is unlikely to be associated with N shortage (*30*), given the overabundance of N in this region (*9*). Broader stoichiometric constraints, soil microbial feedback (*18*, *31*), or counteracting ambient ozone (*6*, *32*) may hold answers. In summary, we find no evidence that current CO_2 concentrations are limiting tree growth in this tallest forest studied so far.

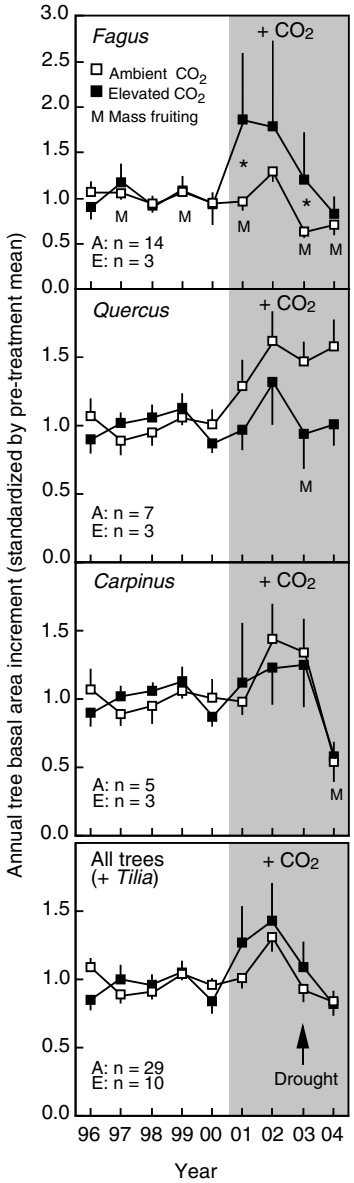

Fig. 4. Stem basal area (BA) growth of trees, standardized by the mean pretreatment BA increment for each tree. The two asterisks in *Fagus* indicate the only significant stimulation; 2003 was a drought summer; no significant $CO_2 \times$ year interaction in any species). The bottom diagram is for all trees that showed measurable growth, i.e., 10 in elevated CO_2 versus 29 controls (means ± SE).

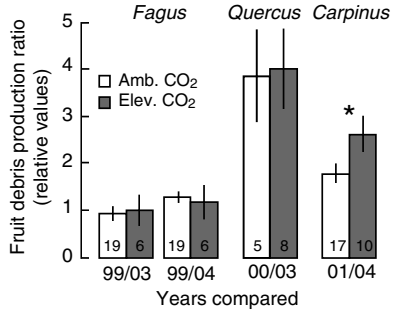

Fig. 5. Fruit debris collected with litter traps for three different tree species in years of mass fruiting. Pretreatment-to-treatment ratios for years with mass fruiting were used to account for spatial variability (numbers within bars for *n* traps). For *Fagus* we show the two latest mass fruiting episodes. The height of the bars denotes the degree of mass fruiting in the respective years ($P = 0.047$ for *Carpinus* in 2004; mean ± SE).

Table 2. Change in litter composition (% dry matter) as well as 220-day decomposition of fresh leaf litter collected in the canopy (two-factorial ANOVA with CO_2 and species as fixed factors).

Litter trait	Difference (%)	*P*-value	Main driver
Total C	+2	0.03	Nonspecific
Total N	−1	0.73	−
NSC*	+21	0.002	*Fagus*, TAP†
Lignin‡	−11	0.007	*Fagus*, TAP
Litter mass loss	+14	0.001	Carpinus, TAP

*Nonstructural carbohydrates (sugar, starch). †TAP is a mix of rare taxa of the genera *Tilia*, *Acer*, and *Prunus*. ‡Correcting for NSC-free litter mass changed the relative difference in lignin between CO_2 treatments from −10.7 to −10.0% ($P = 0.01$) but had no influence on the relative difference in nitrogen concentration.

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Fig. S1

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Community Structure of Corals and Reef Fishes at Multiple Scales

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Distributions of numerical abundance and resource use among species are fundamental aspects of community structure. Here we characterize these patterns for tropical reef fishes and corals across a 10,000-kilometer biodiversity gradient. Numerical abundance and resource-use distributions have similar shapes, but they emerge at markedly different scales. These results are consistent with a controversial null hypothesis regarding community structure, according to which abundance distributions arise from the interplay of multiple stochastic environmental and demographic factors. Our findings underscore the importance of robust conservation strategies that are appropriately scaled to the broad suite of environmental processes that help sustain biodiversity.

Two fundamental aspects of ecosystem structure concern how individuals and resources are partitioned among species (*1*–*5*). Empirically, relative abundances of species approximate either log-series or log-normal distributions. Under the log-series distribution, the number of species is a decreasing function of abundance, with species being most likely to be represented by a single individual (*6*). In contrast, under the log-normal distribution, few species have either very low or very high abundance: On a logarithmic scale, abundance distributions can even exhibit a slight left skew (*7*–*10*). Because the prevalence of extremely rare species differs markedly between these distributions, understanding whether and how communities differ in or shift among these distributions is critically important for predicting consequences of habitat loss and environmental degradation for biodiversity and ecosystem functioning (*11*, *12*).

Several theories have been proposed to account for this variability in shapes of abundance distributions. In particular, niche-based models produce log-series to left-skewed log-normal distributions under different rules for resource allocation (*13*); demographic models can encompass the same range of distributions through changes in rates of dispersal among habitat patches, or the process by which new species originate (*12*, *14*–*17*). Alternatively, a classical null hypothesis asserts that abundance distributions in ecological communities are ubiquitously log-normal (*18*, *19*) and that apparent deviations from this shape arise largely from sampling effects (*7*, *8*, *10*).

Here we examine community structure in scleractinian corals and reef fishes from the family Labridae (wrasses and parrotfishes) at 100 sites arrayed along a 10,000-km transect extending longitudinally from the Central Indo-Pacific global biodiversity hot spot to the comparatively depauperate reefs of French Polynesia (Fig. 1). The rapid, ongoing, and worldwide decline of coral reefs has made it an urgent priority to understand the processes that structure and sustain these ecosystems (*20*–*22*). Scleractinian corals and labrid fishes are principal structure-formers and major consumers, respectively, of coral reefs, so understanding assemblage structure for these groups is particularly relevant to predicting and managing the consequences of biodiversity loss (*22*, *23*). We assess these patterns in local communities from three habitats (reef slope, crest, and flat), using an explicitly hierarchical design that allows us to assess how numerical abundance and resource-use distributions change across three spatial scales: the "local community" scale (tens of meters); the scale of clusters of nearby reefs [here termed the "reef" scale (kilometers)]; and the "metacommunity" scale (tens to hundreds of kilometers) (*24*). We quantify numerical abundance distributions by counts of individual fishes and coral colonies, and we estimate species' population biomass (for fishes) and percentage cover (for corals) as proxies for resource use (fig. S1). To overcome persistent problems with the power of goodness-of-fit and model selection statistics in analyses of abundance distributions (*19*, *25*, *26*), we apply contemporary analytical techniques derived from information theory (*24*).

Numerical abundance and resource-use distributions exhibit markedly different changes in shape with increasing scale, differences that are strikingly parallel in fishes and corals. Fish biomass and coral cover are very closely approximated by log-normal distributions at all scales (Fig. 2 and fig. S2). In contrast, both fishes and corals exhibit a log-series–like distribution of numerical abundance at the local community scale, with decreasing numbers of species in each successive octave (Fig. 3A). However, an alternative hypothesis is that the underlying numerical abundance distribution is log-normal in shape, but that it is truncated because the rarest species have not been sampled (the "veil effect") (*7*, *8*). We find overwhelming support for the latter hypothesis: The truncated log-normal distribution fits the data better than the log-series distribution, with >99% confidence (Fig. 3A and table S1). Further support for a log-normal distribution emerges as data are pooled at the reef and metacommunity scales and subsequently pooled across habitats within metacommunities: The truncated log-normal distribution continues to exhibit excellent fit to the data; the mode of the abundance distribution shifts toward the right; and the distribution becomes less truncated, consistent with the "unveiling" of a log-normal distribution of numerical abundance with increasing sampling effort (Fig. 3B and fig. S3). Moreover, the size of the metacommunity species pool, when estimated from the best-fit log-normal parameters, agrees closely with predictions based on the nonparametric jackknife species richness estimator (Fig. 4).

These results indicate that numerical abundance and resource use both have log-normal distributions, but that these distributions emerge at markedly different scales for both corals and

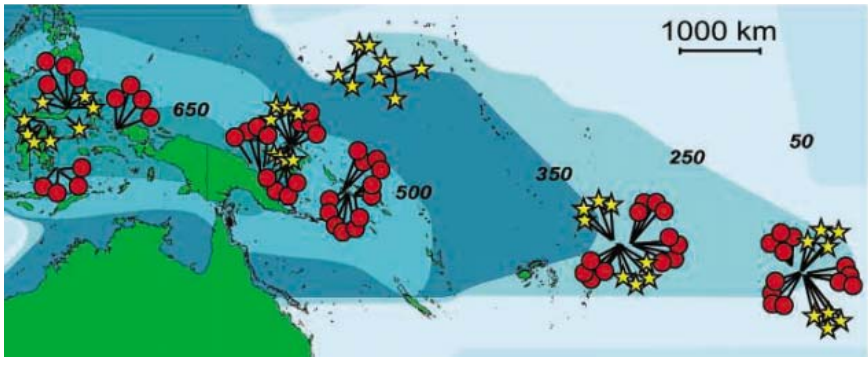

Fig. 1. Locations of study sites. Coral sites are identified with red circles, and fish sites are shown as yellow stars. At each site, three local communities were surveyed, one from each of three habitats (slope, crest, and flat), yielding 180 local communities for corals and 120 for fishes (*24*). Contours of fish species richness are shown and labeled in italics to illustrate the transect's biodiversity gradient; a similar gradient is exhibited by coral species (*29*).

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fishes. Our proxies of resource use are, on average, over 99% unveiled at the scale of local communities (that is, less than 1% of the fitted log-normal distribution is unsampled) (Fig. 2). To unveil numerical abundance distributions to a comparable level, sample sizes approximately two orders of magnitude larger would be required (*24*). This scale discrepancy is inconsistent with arguments based on niche theory, according to which the partitioning of numerical abundance and of resources are equivalent processes (*2*). The strikingly parallel results for corals and fishes also argue against a niche-based explanation. Although corals differ in their performance across habitats (along gradients in light, exposure, turbidity, etc.), their potential for resource partitioning within habitats is likely to be constrained because they compete for a relatively small set of limiting resources. In contrast, niche differences within habitats (such as trophic differentiation) among co-occurring labrid fishes are far more extensive. Species within this family exploit almost every available food resource on coral reefs (*27*); thus, they can partition resources along many more environmental axes than corals can.

Our study's support for underlying log-normal distributions of numerical abundance is highly consistent with an ecological null hypothesis for community structure that is based on the Central Limit Theorem (CLT): Log-normal abundance distributions arise as a statistical consequence of multiplicative interactions among a large number of stochastic ecological factors that affect population growth (*18*). Resource acquisition is also likely to have a strong multiplicative component, because the capacity to acquire additional resources is likely to scale with resources already acquired (for example, space acquisition in corals will be proportional to space already occupied). Thus, log-normal distributions of resource use are also consistent with this hypothesis. The CLT hypothesis is controversial, and its logic has been criticized (*26*). However, the hypothesis that log-normal abundance distributions are a general consequence of stochastic variation in population growth rates is consistent with demographic theory that explicitly integrates environmental stochasticity with a general form of density-dependent interactions among species (*15*). This contrasts with models that do not incorporate environmental stochasticity and make more specific assumptions about interspecific density-dependence, which tend to predict a broad family of shapes for abundance distributions (*12*, *16*, *17*).

Acceptance of the generality of log-normal abundance distributions has been hindered by the prevalence of deviations from the log-normal in empirical data. Indeed, numerous alternative theories have been proposed in order to account for such deviations (*12*, *13*, *16*, *17*). Although our data exhibit many of these same deviations, our multiscale analysis indicates that they are likely to be caused by sampling effects, rather than biological processes. For resource-use distributions, a hypothesis that left skew is a statistical artifact of pooling samples from multiple locations (*10*) is supported: A slight left skew emerges as local communities are pooled at larger scales (fig. S2). For numerical abundance, the veil effect is supported by the excellent fit of the truncated log-normal distribution to our local community abundance distributions (Fig. 3A and fig. S3), and our multiscale approach uncovers additional evidence for an underlying log-normal distribution of numerical abundance. Specifically, changes in the shape of the distribution with increasing scale (Fig. 3B) match what is expected if a log-normal distribution is being unveiled. Moreover, metacommunity richness levels predicted by the

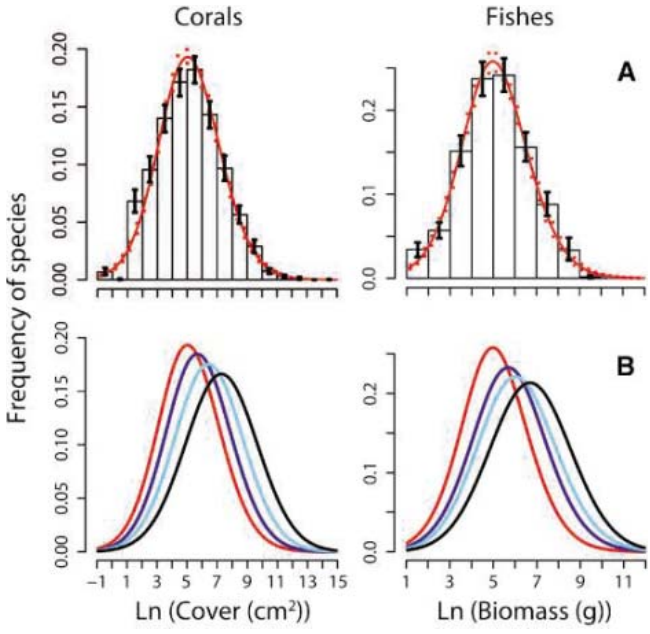

Fig. 2. (**A**) Distributions of resource-use proxies (coral cover and fish biomass) at the local community scale, with best-fit continuous log-normal distributions. Bars represent the mean fraction of observed species in each abundance class. Solid red lines show the mean of best-fit log-normal distributions, normalized for plotting on the same scale as observed frequencies. Black error bars and red dotted lines represent 95% bootstrap confidence limits on observed and predicted means, respectively. (**B**) Consistency in abundance distributions with increasing scale. Best-fit log-normal distributions are plotted for local communities [red line, reproduced from (A)], along with communities pooled at the reef (dark blue line) and metacommunity (light blue line) scales, and finally pooled across habitats at the metacommunity scale (black line).

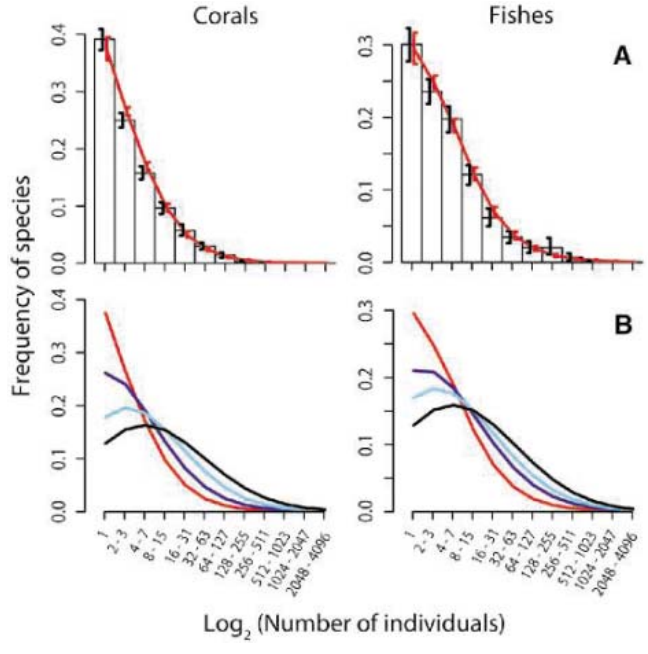

Fig. 3. Numerical abundance distributions for corals and fishes at (**A**) the local community scale, with best-fit Poisson log-normal distributions. These distributions explicitly characterize the veil effect produced by sampling from an underlying log-normal distribution (*24*). Octaves are here plotted as true doubling classes, with each successive octave containing twice as many abundance categories as the preceding one. Bars represent the mean fraction of observed species in each abundance class. Red lines represent the mean of predicted frequencies across local communities, based on independent maximum-likelihood fits of the Poisson log-normal distribution to each local community. Black and red error bars represent 95% bootstrap confidence limits on observed and predicted mean frequencies, respectively. (**B**) Unveiling a log-normal abundance distribution. Best-fit Poisson log-normal distributions are plotted for local communities [red line, reproduced from (A)], communities pooled at the reef (dark blue line) and metacommunity (light blue line) scales, and finally pooled across habitats at the metacommunity scale (black line).

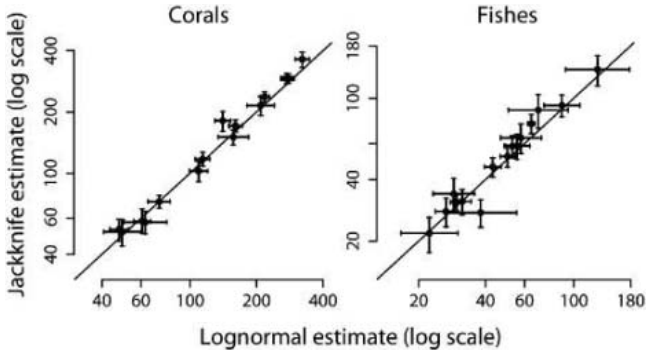

Fig. 4. Comparison of the size of the metacommunity species pool, as predicted by the Poisson log-normal distribution (horizontal axis) and the jackknife estimator (vertical axis), with standard errors (*24*). The diagonal line is the unity line (log-normal estimate = jackknife estimate). Each point corresponds to one habitat type within each metacommunity. The two sets of estimates exhibit excellent fit to the unity line (r^2 = 0.98 and 0.94 for corals and fishes, respectively).

log-normal model agree with predictions from the nonparametric jackknife estimator, which makes no assumptions about the form of the underlying distribution of numerical abundance (Fig. 4).

The search for a limited suite of processes that accounts for consistent patterns in species' relative abundances has occupied ecologists for at least a half century (*1*); this search has accelerated as the worsening biodiversity crisis has focused attention on the need to understand how high-diversity communities are structured (*12*, *13*). Our results lend strong support to a classical, but controversial, null hypothesis regarding community structure: The shape of species-abundance distributions arises as a general consequence of environmental stochasticity, through its effects on population dynamics. This finding underscores the importance of robust conservation strategies that adequately encompass the spectrum of environmental variability to which coral reef organisms are exposed. Accordingly, conservation efforts should expand in scale and scope, moving beyond localized protected areas and toward a seascape approach (*28*). Given the accelerating pace of coral reef habitat loss worldwide (*21*), addressing this challenge remains an urgent priority.

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References and Notes

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Global Patterns of Predator Diversity in the Open Oceans

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The open oceans comprise most of the biosphere, yet patterns and trends of species diversity there are enigmatic. Here, we derive worldwide patterns of tuna and billfish diversity over the past 50 years, revealing distinct subtropical "hotspots" that appeared to hold generally for other predators and zooplankton. Diversity was positively correlated with thermal fronts and dissolved oxygen and a nonlinear function of temperature (~25°C optimum). Diversity declined between 10 and 50% in all oceans, a trend that coincided with increased fishing pressure, superimposed on strong El Niño–Southern Oscillation–driven variability across the Pacific. We conclude that predator diversity shows a predictable yet eroding pattern signaling ecosystem-wide changes linked to climate and fishing.

Humans have exploited oceanic predators such as tuna, billfish, sharks, and sea turtles for millennia. Although our knowledge of individual species has rapidly advanced, for example, through sophisticated tagging studies (*1*, *2*), community-wide patterns of abundance and diversity are only beginning to be understood (*3*). This knowledge is timely. Many species have declined, are vulnerable to overfishing, or are threatened by extinction (*4*, *5*), and there is a concern that widespread predator declines can trigger unforeseen ecosystem effects (*6–8*). Effective management and conservation in the open oceans will depend on resolving the spatial distribution of multiple species, ecological communities, and fishing effort (*1*, *2*, *9–11*). Recent studies performed on a regional scale have indicated that predator species may aggregate at distinct diversity hotspots—areas of high species diversity that may represent important oceanic habitats and hold particular value for biodiversity conservation (*3*, *11*). Yet, global-scale patterns and trends of predator diversity have remained obscure. We investigated the global distribution of predator diversity, how it relates to regional oceanography, and whether diversity has changed over time.

As a first step, we used global 5° by 5° Japanese longlining data from 1990 to 1999 to analyze contemporary patterns of tuna and bill-

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fish diversity (*12*). Tuna and billfish are among the most ubiquitous, ecologically important, and economically important oceanic predators, and they range globally from the equator to temperate regions (0 to ~55° latitude). Pelagic longlines are the most widespread fishing gear in the open ocean and are primarily used to target tuna and billfish. These are baited lines of up to 100 km in length that catch a wide range of predators in a similar way, operating across global scales. The Japanese logbook data represents the world's largest longline fleet and the only globally consistent data source, reporting species composition, catch, and effort for all tuna (Thunnini), billfishes (Istiophoridae), and swordfish (Xiphiidae) (table S1). We used statistical rarefaction techniques to standardize for differences in fishing effort among 5° by 5° cells and to estimate two common measures of species diversity: species richness (the expected number of species standardized per n individuals) and species density (the expected number of species standardized per k hooks) in each cell (*12*, *13*). We report results that correspond to the average number of individuals (n = 50) and hooks (k = 1000) in a single longlining set. Alternative parameters (n = 20, 100, and 500; k = 500, 2000, and 5000) gave similar results. The difference between these two diversity indices is that species richness reflects solely the number of species, whereas species density reflects the number of species per unit area (*13*). Whereas richness may be more interesting ecologically, species density is more meaningful for conservation and management.

Tuna and billfish species richness (Fig. 1A) and species density (Fig. 1B) showed a consistent global pattern, indicating peaks of diversity at intermediate latitudes (15 to 30°N or S) and lower diversity toward the poles and at the equator. In the Atlantic and Indian Ocean, diversity also appeared to be higher in western regions as compared with eastern regions. Hotspots of species richness and density were clustered mostly in the subtropics, namely off the U.S. and Australian east coasts, south of the Hawaiian Islands chain, east of Sri Lanka, and most prominently in the southeastern Pacific (Fig. 1C).

We checked the generality of these results using independent scientific observer data from longline fisheries in the Atlantic and Pacific Ocean (Fig. 1, D to F). Observer records were collected by U.S. and Australian management agencies from 1990 to 1999 (*12*). They have much better taxonomic breadth (N = 145 species, including tuna, billfish, other bony fishes, sharks, pelagic rays, whales, dolphins, turtles, and large seabirds) but much smaller geographic range (<10%) and sample sizes (<1%) compared with the global Japanese data (table S1). Total predator richness, as calculated from the observer data, was highly correlated with the Japanese tuna and billfish data (Fig. 1, D to F), which suggests that tuna and billfish may be used to predict total predator diversity. In addition, we found a strong correlation between tuna and billfish richness with foraminiferan zooplankton diversity (Fig. 1G). Before this study, foraminifera were the only oceanic species for which global diversity patterns were known (*14*). Remarkably, these single-celled organisms show the same latitudinal distribution as large predators, with distinct diversity peaks at intermediate latitudes (*14*). Similar patterns appear to hold for other zooplankton groups (*14*). This suggests that the global pattern of diversity shown here could be general across several trophic levels and different from latitudinal patterns on land, where diversity usually peaks around the equator (*15*).

Asking what oceanographic variables may explain global patterns of predator diversity, we explored the effects of remotely sensed sea surface temperatures (SST) (mean and spatial gradients), dissolved oxygen levels, eddy kinetic energy (calculated from sea surface height anomalies), chlorophyll *a* (mean and spatial gradients), and depth (mean and spatial gradients) on diversity, using spatial regression models (Table 1, Fig. 2, and fig. S1). These models accounted for spatial covariance among cells, while testing the relations between oceanographic variables and diversity (*12*). Most variables have been suggested to explain the distributions of individual predator species (*16*), but their effects on diversity had been unknown. Stepwise elimination of nonsignificant variables (eddy kinetic energy, depth, chlorophyll, all $P > 0.1$) revealed mean temperature, SST gradients, and oxygen as main factors (Table 1). Spatial covariance parameters indicated that latitudinal variation was much more pronounced than longitudinal variation (Table 1). Adjacent cells were prac-

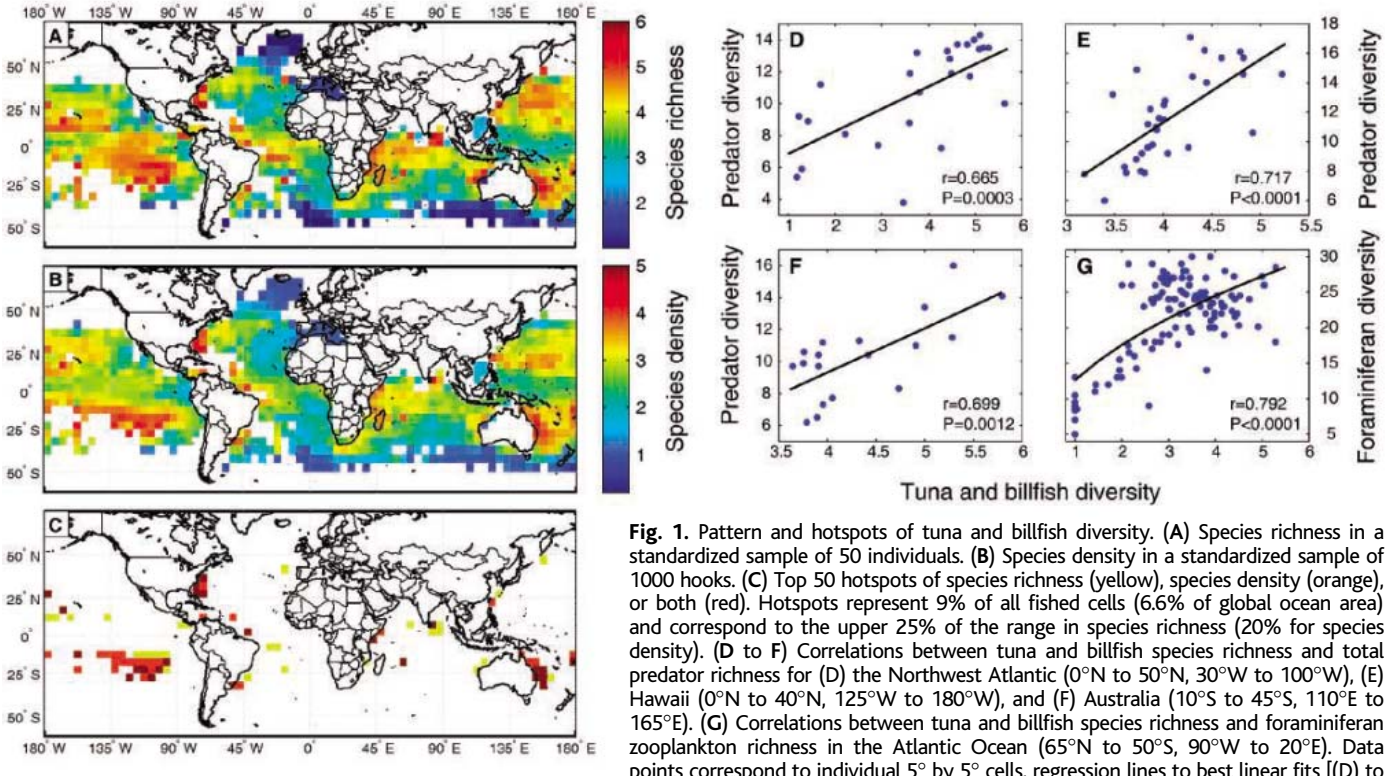

Fig. 1. Pattern and hotspots of tuna and billfish diversity. (**A**) Species richness in a standardized sample of 50 individuals. (**B**) Species density in a standardized sample of 1000 hooks. (**C**) Top 50 hotspots of species richness (yellow), species density (orange), or both (red). Hotspots represent 9% of all fished cells (6.6% of global ocean area) and correspond to the upper 25% of the range in species richness (20% for species density). (**D** to **F**) Correlations between tuna and billfish species richness and total predator richness for (D) the Northwest Atlantic (0°N to 50°N, 30°W to 100°W), (E) Hawaii (0°N to 40°N, 125°W to 180°W), and (F) Australia (10°S to 45°S, 110°E to 165°E). (**G**) Correlations between tuna and billfish species richness and foraminiferan zooplankton richness in the Atlantic Ocean (65°N to 50°S, 90°W to 20°E). Data points correspond to individual 5° by 5° cells, regression lines to best linear fits [(D) to (F)] or log-linear fits (G).

tically uncorrelated across latitude but were spatially correlated across 10° to 15° longitudinal bands.

SST (Fig. 2A) clearly emerged as the strongest predictor of species richness and species density (Table 1), showing a positive correlation over most of the observed range (5°C to 25°C) but a negative trend above 27°C mean SST (Fig. 2, D and E). For example, species richness was depressed around cool upwelling regions in the eastern Atlantic and Pacific, but also in the western tropical Pacific "warm pool" (Fig. 1A), which shows exceptionally high temperatures (>30°C) (Fig. 2A). A third-order polynomial model of SST produced the best fit for global predator diversity (Table 1 and Fig. 2, D and E). A very similar model of SST explained spatial variation in foraminiferan zooplankton diversity (*14*). For foraminifera, the decline of diversity at high temperatures was linked to reduced niche availability due to a sharp, shallow thermocline in the tropical ocean (*14*). For tuna, which generate large amounts of metabolic heat, physiological mechanisms such as overheating at high ambient temperatures could play an additional role.

Much of the observed variation in species richness and density around the general SST trend was well explained by synoptic spatial temperature gradients (Fig. 2, B, D, and E). Sharp temperature gradients (indicated by yellow and red, Fig. 2, B, D, and E) indicate frontal zones and warm- or cold-core eddies that are associated with mesoscale oceanographic variability. Fronts and eddies often attract large numbers of species such as seabirds (*17*), tuna (*16*), turtles (*2*), billfish, and whales (*18*), likely because they concentrate food supply, enhance local production, and increase habitat heterogeneity (*10*, *19*). Persistent fronts also form important landmarks along transoceanic migration routes (*20*). Our analysis implies that these regional habitat features may also be important for global diversity.

Oxygen concentrations were positively correlated with diversity (Fig. 2C and Table 1). This is likely to relate to species physiology, because low oxygen levels (<2 ml l^{-1}) may limit the cardiac function and depth range of many tuna species (*16*). Regions of low oxygen are located west of Central America, Peru, and West Africa and in the Arabian Sea (Fig. 2C). Indeed, despite optimal SST around 25°C, most of these areas showed conspicuously low diversity (Fig. 1, A and B).

We analyzed temporal trends of tuna and billfish diversity since 1952, when industrial exploitation of the open oceans first expanded globally. Using recently derived correction factors for each species (*21*), we standardized

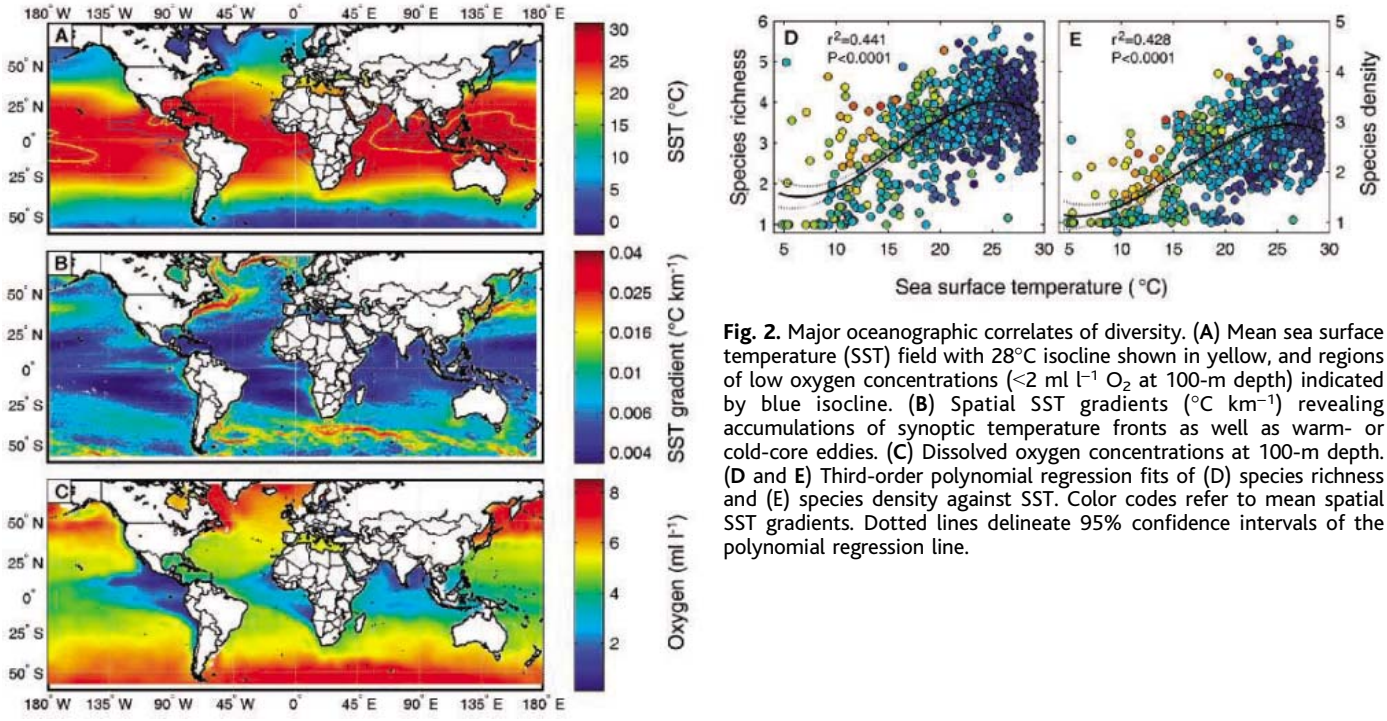

Fig. 2. Major oceanographic correlates of diversity. (**A**) Mean sea surface temperature (SST) field with 28°C isocline shown in yellow, and regions of low oxygen concentrations (<2 ml l^{-1} O_2 at 100-m depth) indicated by blue isocline. (**B**) Spatial SST gradients (°C km^{-1}) revealing accumulations of synoptic temperature fronts as well as warm- or cold-core eddies. (**C**) Dissolved oxygen concentrations at 100-m depth. (**D** and **E**) Third-order polynomial regression fits of (D) species richness and (E) species density against SST. Color codes refer to mean spatial SST gradients. Dotted lines delineate 95% confidence intervals of the polynomial regression line.

Table 1. Spatial regression model relating tuna and billfish diversity to oceanography. Regression coefficients and their standard error estimates (SE) are given, along with test statistics. Covariance parameters estimate spatial correlation among 5° by 5° cells, assuming an anisotropic exponential decay model: $\mathrm{cov}(y_i, y_j) = \sigma^2 \exp - (\theta_1 d_{i,j,1} + \theta_2 d_{i,j,2})$, where θ_1 describes the latitudinal and θ_2 the longitudinal covariance parameter, $d_{i,j,1}$ the latitudinal distance, and $d_{i,j,2}$ the longitudinal distance between cells y_i and y_j.

Variable	Species richness		t	P	Species density		t	P
	Coefficient	SE			Coefficient	SE		
Intercept	1.694	0.846	2.0	0.1833	0.887	0.630	1.4	0.2945
Sea surface temperature	–0.443	0.153	–2.9	0.0038	–0.342	0.115	–3.0	0.0030
$(SST)^2$	0.04	0.009	4.3	<0.0001	0.030	0.007	4.3	<0.0001
$(SST)^3$	–0.001	0.0002	–4.5	<0.0001	–0.001	0.0002	–4.4	<0.0001
SST gradient	48.69	13.7	2.9	0.0042	29.617	9.830	3.0	0.0027
Dissolved oxygen	0.166	0.0495	3.4	0.0008	0.173	0.039	4.5	<0.0001
Covariance parameters	θ_1	θ_2	σ^2		θ_1	θ_2	σ^2	
Estimates	0.242	0.089	0.475		0.179	0.071	0.267	
Likelihood ratio test	df = 2	$X^2 = 64.4$	$P < 0.0001$		df = 2	$X^2 = 139.7$	$P < 0.0001$	

Japanese longline data for historical changes in fishing practices, specifically the increase in longline depth during the 1970s and 1980s to target deeper swimming species such as bigeye tuna (*Thunnus obesus*). Species richness and species density were calculated from these data by rarefaction, as outlined above. Resulting data sets are displayed in Movies S1 and S2. To extract seasonal, interannual, and decadal trends, we estimated changes in average species richness and species density across the Atlantic (Fig. 3A), Indian (Fig. 3B), and Pacific Oceans (Fig. 3C), using linear mixed effects models that accounted for spatial autocorrelation and for changes in the spatial and seasonal coverage of fished cells (*12*).

Results indicated that interannual variation was an order of magnitude stronger than seasonal variation (table S2). Species richness showed pronounced year-to-year fluctuations and decadal declines of 10% to 20% in all oceans (Fig. 3, A and C). However, this pattern reversed in the Pacific in 1977, when richness began to increase again to pre-exploitation levels (Fig. 3C). Species density showed gradual ~50% declines in the Atlantic (Fig. 3A) and Indian Oceans (Fig. 3B) and ~25% decline in the Pacific (Fig. 3C). These declines were most pronounced in intensely fished tropical areas, particularly in the Indian and Atlantic Oceans (Movies S1 and S2). The trajectories of species richness and, particularly, species density were negatively correlated with 5- to 10-fold increases in total catch of tuna and billfish in all oceans since 1950 (Fig. 3, A to C), which may have led to regional depletion of vulnerable species (*4*, *5*, *22*). Larger declines in species density likely result from the combined effects of decreasing richness and decreasing abundance over time. Although strong temporal autocorrelation in these trends precluded statistical inference, we could not identify a factor other than fishing that may plausibly explain long-term, global-scale declines. Gradual ocean warming, for example, may lead to increased diversity over most of the observed range (Fig. 2, D and E, and results below), although the effects of complex changes in current patterns and regional oceanography are hard to predict. To explore historic trends in temperature and to test the predictive value of our oceanographic model (Table 1) over time, we fitted it to depth-corrected diversity and SST data from the 1960s, 1970s, 1980s, and 1990s. We found that relations among sea surface temperature, SST gradients, oxygen, and diversity were very similar across the last four decades (table S3). These findings may suggest that there were no major decadal changes in the relations between diversity and oceanography.

Short-term variability in species richness, however, appeared to be linked to climate, at least in the Pacific. Pronounced year-to-year changes in species richness D (expressed as first difference $\Delta_t = D_{t+1} - D_t$) showed a strong positive correlation ($r = 0.54$, $P < 0.0001$) with the El Niño–Southern Oscillation (ENSO) index (Fig. 3D). When we analyzed this pattern spatially at the level of individual cells, we saw that widespread increases in species richness during El Niño warm phases occurred across the North and South Central Pacific (Fig. 3E). This could be linked to regional warming and concomitant changes in recruitment or movement of species into these areas; changes in catchability could also be a factor. Substantial decreases in species richness were seen in the tropical Eastern Pacific, a region that suffers from greatly reduced productivity and associated mass mortality of marine life during El Niño (Fig. 3E). Indeed, we found that several large predator species (such as Indo-Pacific blue marlin, *Makaira mazara*) (Fig. 3F) showed lower abundance in the tropical Eastern Pacific and apparent increases across the North and South Central Pacific with increasing El Niño conditions. This is probably not an effect of changing catchability. Surface-dwelling species such as marlins would likely extend their vertical range with the deepening of the thermo- and oxyclines during El Niño events in the Eastern Pacific. Hence, they would become more catchable because they are more likely to intercept longlines, which are currently set at a mean depth of ~100 m and a maximum of 400 m (*21*). The observed pattern (Fig. 3F) is the opposite and is more consistent with ENSO-induced migration of highly mobile predators to favorable areas. This hypothesis may be tested further using satellite tracking data currently being gathered for a wide range of Pacific predators (*23*).

In the Indian Ocean, ENSO or the Indian Ocean Dipole Index (*12*) did not appear to influence species richness or density ($P > 0.2$). In the Atlantic, however, we detected a weak positive trend of species richness with the North Atlantic Oscillation Index (NAO) ($r = 0.301$, $P = 0.062$) and, in the Pacific, a significant correlation of species richness with the Pacific Decadal Oscillation Index (PDO) ($r = 0.306$, $P = 0.035$), which may be linked to long-term changes in the ENSO regime (*24*).

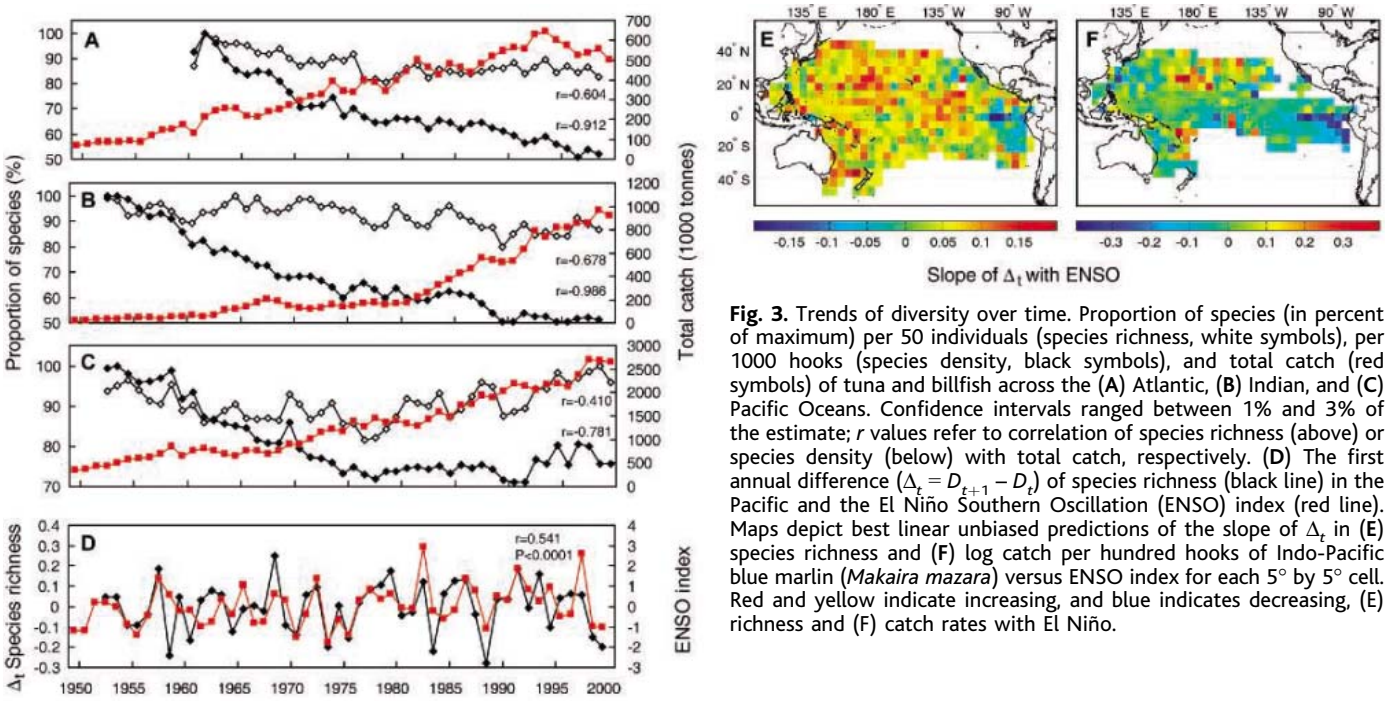

Fig. 3. Trends of diversity over time. Proportion of species (in percent of maximum) per 50 individuals (species richness, white symbols), per 1000 hooks (species density, black symbols), and total catch (red symbols) of tuna and billfish across the (**A**) Atlantic, (**B**) Indian, and (**C**) Pacific Oceans. Confidence intervals ranged between 1% and 3% of the estimate; *r* values refer to correlation of species richness (above) or species density (below) with total catch, respectively. (**D**) The first annual difference ($\Delta_t = D_{t+1} - D_t$) of species richness (black line) in the Pacific and the El Niño Southern Oscillation (ENSO) index (red line). Maps depict best linear unbiased predictions of the slope of Δ_t in (**E**) species richness and (**F**) log catch per hundred hooks of Indo-Pacific blue marlin (*Makaira mazara*) versus ENSO index for each 5° by 5° cell. Red and yellow indicate increasing, and blue indicates decreasing, (E) richness and (F) catch rates with El Niño.

When the PDO suddenly reversed to a warm phase in 1977, famously inducing a basin-wide regime shift (*25*), the trajectory of predator species richness also reversed (Fig. 3C).

Although other studies have confirmed the effects of climate perturbations on movement and recruitment of individual species (*25*, *26*), this is the first account of any ocean-wide changes in community diversity. We suggest that our results reconcile the different effects of fishing and climate, a matter of intense debate (*27*). We propose that fishing may primarily drive long-term, low-frequency variation in fish communities through gradual changes in species abundance, composition, and size (*28*), whereas climate induces year-to-year variation that may modify decadal trends only in cases where lasting regime shifts occur (*25*).

These results establish a dynamic global pattern of tuna and billfish diversity through space and time. Detailed data for other predators and zooplankton strongly suggest that at least the spatial pattern could be general across taxonomically distant species groups. However, we caution that there are likely some important exceptions. Marine mammals, for example, may show high seasonal diversity in subpolar regions, such as the Bering Sea (*7*). Also, the coarse resolution of our data may mask smaller scale (<100 km) variation associated, for example, with coastal habitats or seamounts (*11*). More work is needed to resolve such variation, particularly in nearshore regions. At the global scale examined here, sea surface temperature, SST gradients, and oxygen were consistently correlated with species diversity across at least four decades. Optimal habitats that attract numerous species appeared to be characterized mainly by warm waters (~25°C) with sufficient oxygen concentrations (>2 ml l^{-1}) in combination with mesoscale oceanographic gradients, resulting in the formation of feeding habitats around thermal fronts and eddies. Fine-scale experimental studies confirm the importance of temperature and oxygen levels, as well as food concentrations for single predator species (*2*, *9*, *16*, *29*). Here, we have shown that these variables also correlate with global diversity patterns, despite large regional differences in environmental conditions and species identities.

These results can be used to inform conservation and management of the high seas. First, species richness and density as calculated from standardized longlining data appear to be sensitive indicators of community-wide changes that may integrate the effects of both fishing and climate as major agents of change. Second, knowledge of global diversity patterns, when merged with fine-scale information on habitat use, spawning areas, migration patterns, and fishing mortality (*1*, *2*, *9*), could be used to define priority areas for ocean conservation. Current conservation efforts such as the international High-Seas Marine Protected Area Initiative (*30*) may thereby direct limited resources efficiently and maximize conservation benefits for the future. These efforts appear even more urgent when considering ocean-scale declining trends in predator diversity as seen in our data. We caution that these trends are based on common target species. Incorporating information on vulnerable bycatch species such as sharks (*22*) and sea turtles (*2*) might prove these estimates conservative.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1113399/DC1
Materials and Methods
Fig. S1
Tables S1 to S4
Movies S1 and S2
References

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Nuclear Reprogramming of Somatic Cells After Fusion with Human Embryonic Stem Cells

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We have explored the use of embryonic stem cells as an alternative to oocytes for reprogramming human somatic nuclei. Human embryonic stem (hES) cells were fused with human fibroblasts, resulting in hybrid cells that maintain a stable tetraploid DNA content and have morphology, growth rate, and antigen expression patterns characteristic of hES cells. Differentiation of hybrid cells in vitro and in vivo yielded cell types from each embryonic germ layer. Analysis of genome-wide transcriptional activity, reporter gene activation, allele-specific gene expression, and DNA methylation showed that the somatic genome was reprogrammed to an embryonic state. These results establish that hES cells can reprogram the transcriptional state of somatic nuclei and provide a system for investigating the underlying mechanisms.

The generation of embryonic stem (ES) cell lines and cloned animals by somatic cell nuclear transfer has demonstrated that the cytoplasm of an oocyte can reprogram the genome of a somatic cell to an embryonic state (*1*, *2*). There is considerable interest in how reprogramming

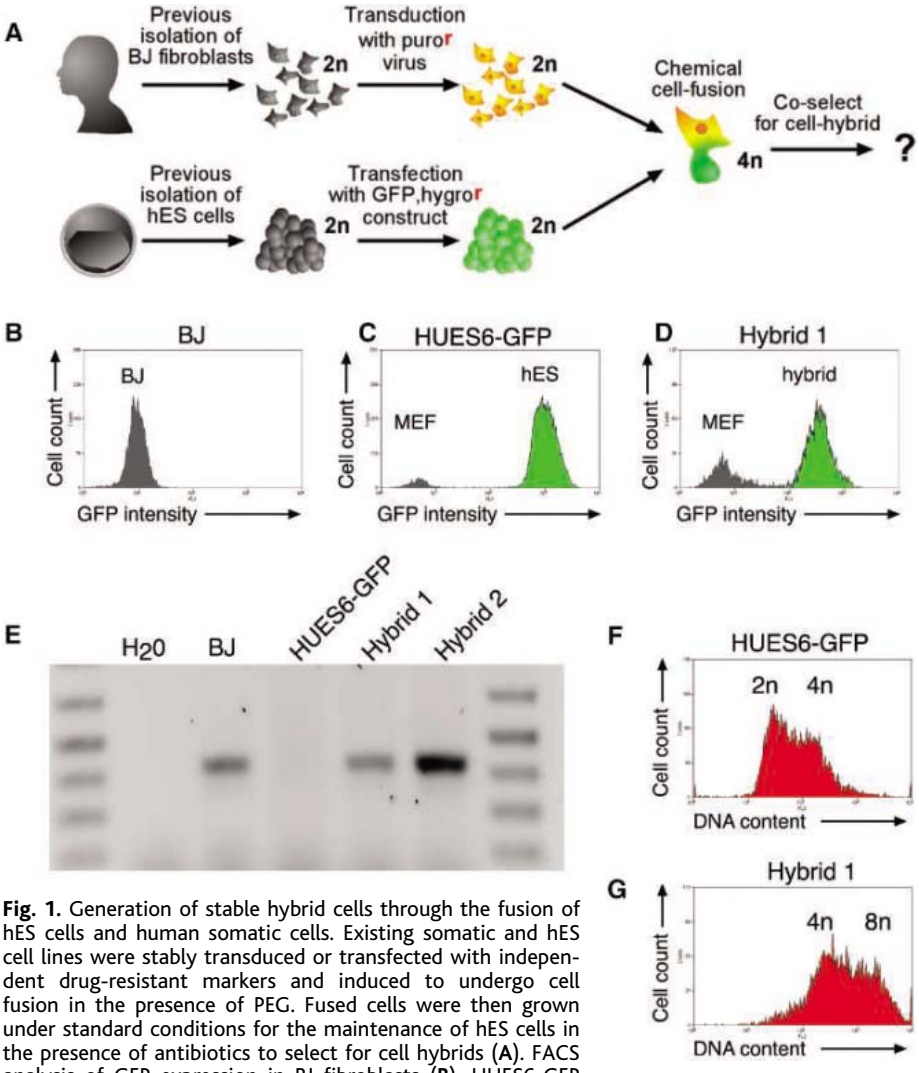

Fig. 1. Generation of stable hybrid cells through the fusion of hES cells and human somatic cells. Existing somatic and hES cell lines were stably transduced or transfected with independent drug-resistant markers and induced to undergo cell fusion in the presence of PEG. Fused cells were then grown under standard conditions for the maintenance of hES cells in the presence of antibiotics to select for cell hybrids (**A**). FACS analysis of GFP expression in BJ fibroblasts (**B**), HUES6-GFP cells (**C**), and hybrid cells (**D**). PCR amplification of DNA sequences specific to the retrovirus used to transduce the somatic BJ fibroblasts (**E**). FACS of HUES6-GFP cells (**F**) and hybrid cells (**G**) stained with propidium iodide.

occurs, because a mechanistic understanding of the process might allow for the direct conversion of adult somatic cells into human ES (hES) cells and thus the production of genetically tailored cell lines for the study and treatment of human disease (*3*–*7*).

On the basis of previous experiments with murine ES cells (*8*, *9*), we reasoned that hES cells might provide an alternative source of material for the reprogramming of human somatic nuclei. To investigate this, we used polyethylene glycol (PEG) to fuse hES cells with human BJ fibroblasts (Fig. 1A) (Materials and Methods). Because of the inefficient nature of cell-fusion (Materials and Methods), we transfected hES cells and retrovirally transduced BJ fibroblasts with independent drug-resistance markers to select for any rare hybrids that might be generated (Fig. 1A). The resulting hygromycin-resistant, green fluorescent protein (GFP)–positive hES cells (HUES6-GFP) and puromycin-resistant BJ fibroblasts were mixed in the presence of PEG and subjected to dual drug selection under conditions used for maintenance of hES cells (*10*). After 10 days of selection, 12 (± 3, $N = 3$) individual colonies of resistant cells were observed. Two typical colonies were picked, expanded, and enzymatically passaged several times before further analysis.

To confirm that these drug-resistant cells arose through cell fusion, we assayed genetic markers carried by the two fusion partners. Fluorescence-activated cell sorting (FACS) analysis demonstrated that, similar to HUES6-GFP cells (Fig. 1C), resistant cells expressed GFP (Fig. 1D). With the use of polymerase chain reaction (PCR), we determined that drug-resistant cells also contained the viral vector introduced into the BJ fibroblasts (Fig. 1E). Consistent with the notion that these cells arose by fusion, they contained twice the relative DNA content of the HUES6-GFP cells (Fig. 1F, 1G). Analysis of chromosome spreads from one of the hybrid cell lines indicated that it consisted predominantly of cells containing 92 chromosomes (fig. S1A). These results demonstrated that, after cell fusion of an hES cell and a somatic cell, stable cell hybrids were produced that contained both the somatic and hES cell chromosomes in a single nucleus (fig. S1B).

In the context of these stable hybrid cells, we addressed the question of whether the somatic genome had been reprogrammed to an embryonic state. If so, then hybrid cells should have a phenotype similar to the parental hES cells. Hybrid cells grew in tight, phase-bright clusters comparable in appearance to hES cells and unlike the spindle-shaped fibroblasts (*11*) (Fig. 2A). Analysis of the DNA content (Fig. 1G) of hybrid cells suggested that their cell-cycle characteristics were similar to hES cells (Fig. 1F). We passaged the hybrid cell lines more than 50 times (>120 population doublings), demonstrating they also share the immortal growth characteristics of hES cells. Furthermore, hybrid cells expressed several markers characteristic of hES cells, including the OCT4 transcription factor (Fig. 2B), alkaline phosphatase activity (fig. S2A), telomerase activity (fig. S3A), and the embryonic-specific antigens SSEA4 (fig. S2B), TRA1-61 (fig. S2C), and TRA1-80 (fig. S2D) at concentrations similar to those found in HUES6-GFP cells (*10*–*16*). In contrast, the somatic BJ cells did not exhibit these characteristics.

To determine whether the hybrid cells display the developmental pluripotency of hES cells, we assessed their ability to differentiate in vitro and in vivo (*10*, *11*, *17*). When cultured in suspension, both hybrid cell lines formed embryoid bodies (EBs), and they formed teratomas after injection into nude mice. Immunostaining showed that both a teratoma (Fig. 2, C to E) and EBs (fig. S3, B to D) contained cells expressing βIII-tubulin (neurectoderm) (*18*), muscle-specific myosin (mesoderm) (*19*), and alpha-fetoprotein (endoderm) (*20*).

To investigate whether transcription of embryonic genes was reactivated in the somatic chromosomes, we used a transgenic reporter in which GFP expression was dependent on promoter elements from the murine *Rex-1* gene (*21*). *Rex-1* is a retinoic acid–regulated zinc-finger protein expressed in ES cells [as well as preimplantation mouse embryos and spermatocytes (*21*)]. This reporter was active when transfected into HUES6 hES cells (Fig. 3A) but inactive after introduction into BJ fibroblasts (Fig. 3B) (*21*). When BJ cells car-

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rying the reporter were fused to GFP-negative HUES6 hES cells (Fig. 3C), the resulting hybrids (Fig. 3D) expressed GFP at a concentration similar to that of HUES6 cells transfected with the same reporter.

We demonstrated that endogenous genes regulating developmental pluripotency were transcribed from the somatic genome by carrying out allele-specific expression analysis. The *CRIPTO/TDGF1* gene product functions as a component of the *NODAL* signaling pathway and is expressed in hES cells but not BJ fibroblasts (Fig. 3E and table S4). An expressed single nucleotide polymorphism specific to the BJ cells was identified in the 3′ untranslated region of the *CRIPTO/TDFG1* transcript (fig. S3E) and assayed in hybrid cells. Three out of nine cDNAs isolated from hybrid cells contained the BJ-specific polymorphism, indicating that the somatic alleles of this gene were activated after cell fusion (fig. S3F).

To determine whether somatic and embryonic transcriptional states coexisted within the hybrid cells or whether the embryonic program predominated, we performed genome-wide transcriptional profiling. The variance in transcriptional profiles between each replicate and the various cell lines was calculated as a Pearson correlation coefficient (PCC) and assessed over 54,675 probe sets (table S1). The PCCs revealed that the transcriptional profiles for BJ fibroblasts and HUES6-GFP cells were very different [PCC = 0.780, range from 0.768 to 0.789, n = 9], whereas the transcriptional profiles of the HUES6-GFP ES cells and the two independent hybrid cell lines varied minimally (HUES6 versus Hybrid1, PCC = 0.985, range from 0.979 to 0.989, n = 9; HUES6 versus Hybrid2, PCC = 0.984, range from 0.978 to 0.989, n = 9). Indeed, the variance between the HUES6-GFP ES cell line and the two hybrid cell lines was less than that we and others observed between independently derived hES cell lines (HSF-6B versus HSF-1B, PCC = 0.978) (table S1) (*22*).

Pairwise comparisons of the male BJ cells and the female HUES6-GFP hES cells showed that 3867 transcripts had values of expression at least twofold higher in the BJ fibroblasts. Only 12 of these somatic-specific transcripts retained their higher level of expression in the cell-hybrids (Fig. 3, G and H) (table S2). Five of these 12 were linked to the Y chromosome, suggesting that their expression values reflected the acquisition of a Y chromosome from the BJ cells rather than failures in reprogramming (Fig. 3G and table S2). In fact, the Y-linked genes expressed in hybrid cells were also expressed at similar amounts in male hES cell lines (*22*, *23*). Similarly, only 20 of 2521 hES-specific transcripts (two- or more-fold higher in hES than BJ) were expressed in the hybrids at lower amounts than in the hES cells (Fig. 3, G and I, and table S3), and a majority of these had expression values closer to those found in hES cells than those found in BJ cells. Of particular note, pluripotency genes including *OCT4*, *NANOG*, *TDGF1*, and *REX1* (*24*–*27*) were expressed at remarkably similar concentrations in the hES cells and the hybrid cells (table S4). Overall, these data show that after stable cell hybrid formation, somatic-specific genes were silenced across the entire genome, whereas the embryonic program of transcription predominated. Indeed, by this analysis, one can conclude that >99% of the transcripts analyzed are reprogrammed.

We next investigated whether epigenetic information underlying the transcription of pluripotency genes was reprogrammed by analyzing the status of a differentially methylated region in the promoter of the *OCT4* gene (*28*). Consistent with previous results (*28*),

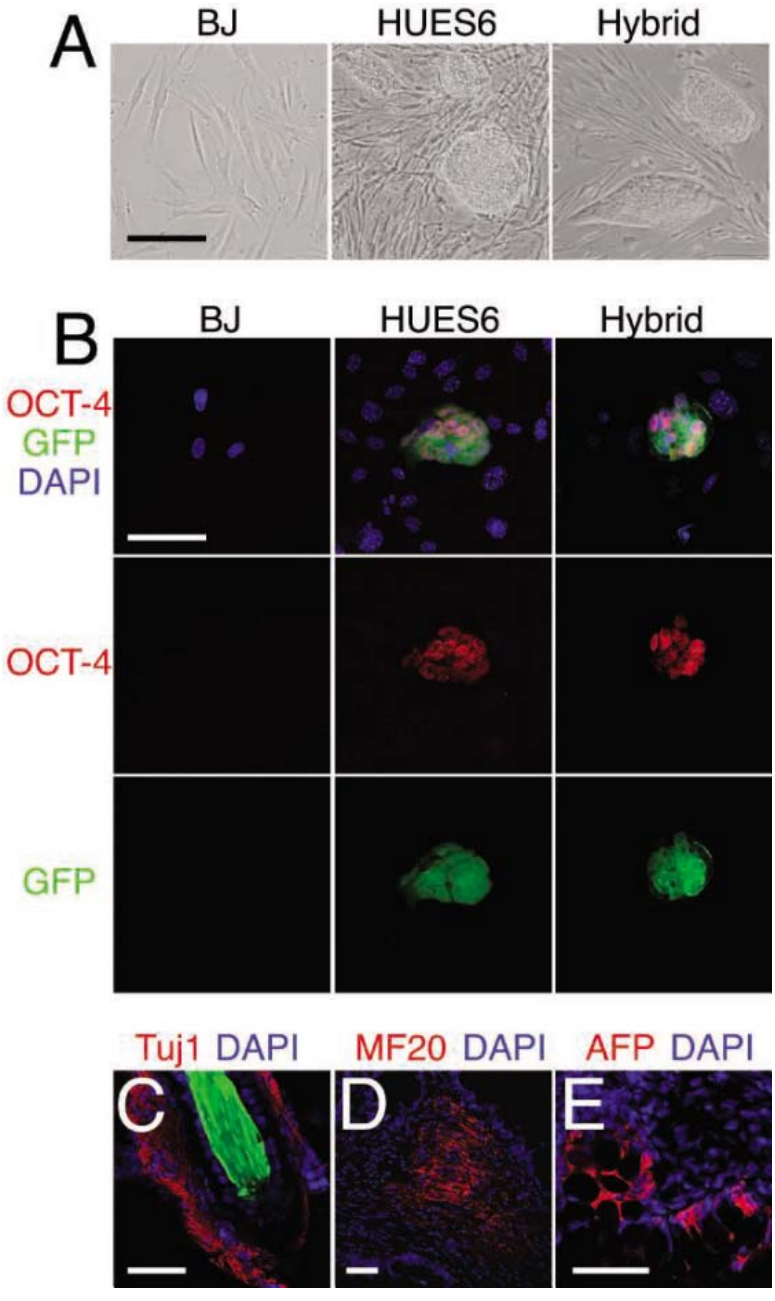

Fig. 2. Hybrid cells can assume an hES cell phenotype. Drug-resistant hybrid cells grew in compact, phase-bright cell clusters with a morphology identical to that of hES cells (**A**). HES cells and hybrid cells expressed the GFP (green) and the transcription factor OCT4 (red), whereas the GFP-negative BJ fibroblasts and mouse embryonic fibroblasts feeder cells did not (**B**). Immunostaining of teratomas derived from hybrid cells revealed the presence of various cell types, including neurons surrounding an ES-derived hair follicle that expressed a neural-specific tubulin (Tuj1, red) (note autofluorescent hair, green) (**C**), skeletal muscle expressing myosin heavy chain (MF20, red) (**D**), and intestinal endoderm expressing the alpha fetal protein (AFP, red) (**E**). Scale bars, 50 μm.

sequencing of bisulfite-modified DNA showed that CpG dinucleotides in this region were generally methylated in somatic BJ cells and unmethylated in hES cells (Fig. 3F). Importantly, when we analyzed DNA from hybrid cells, this region was demethylated and indistinguishable from the epigenetic state found in hES cells (Fig. 3F).

Lastly, we generated stable cell hybrids by fusing another hES cell line, H9 (*10*) with TE76.T pelvic bone cells, demonstrating that the ability of hES cells to reprogram the somatic genome is not restricted to a particular hES cell or somatic cell line (See Supplemental Online Material).

In conclusion, these findings show that hES cells have the capacity to reprogram adult somatic cell chromosomes after cell fusion. HES cells may therefore provide a useful complement to human oocytes for biochemical and genetic studies aimed at understanding how to reprogram differentiated cells to an embryonic state and thereby increase their developmental potential. Eventually, this approach might lead to an alternative route for creating genetically tailored hES cell lines for use in the study and treatment of human disease. However, a substantial technical barrier remains before hES cells could be used for therapeutic purposes: specifically, the elimination of the ES cell chromosomes either before or after cell fusion (*18*). If hES cell enucleation can be performed without the loss of reprogramming activity, and/or if these fusion studies lead to an understanding of the factors needed for reprogramming, these approaches may circumvent some of the logistical and societal concerns surrounding somatic-cell nuclear transfer into human oocytes.

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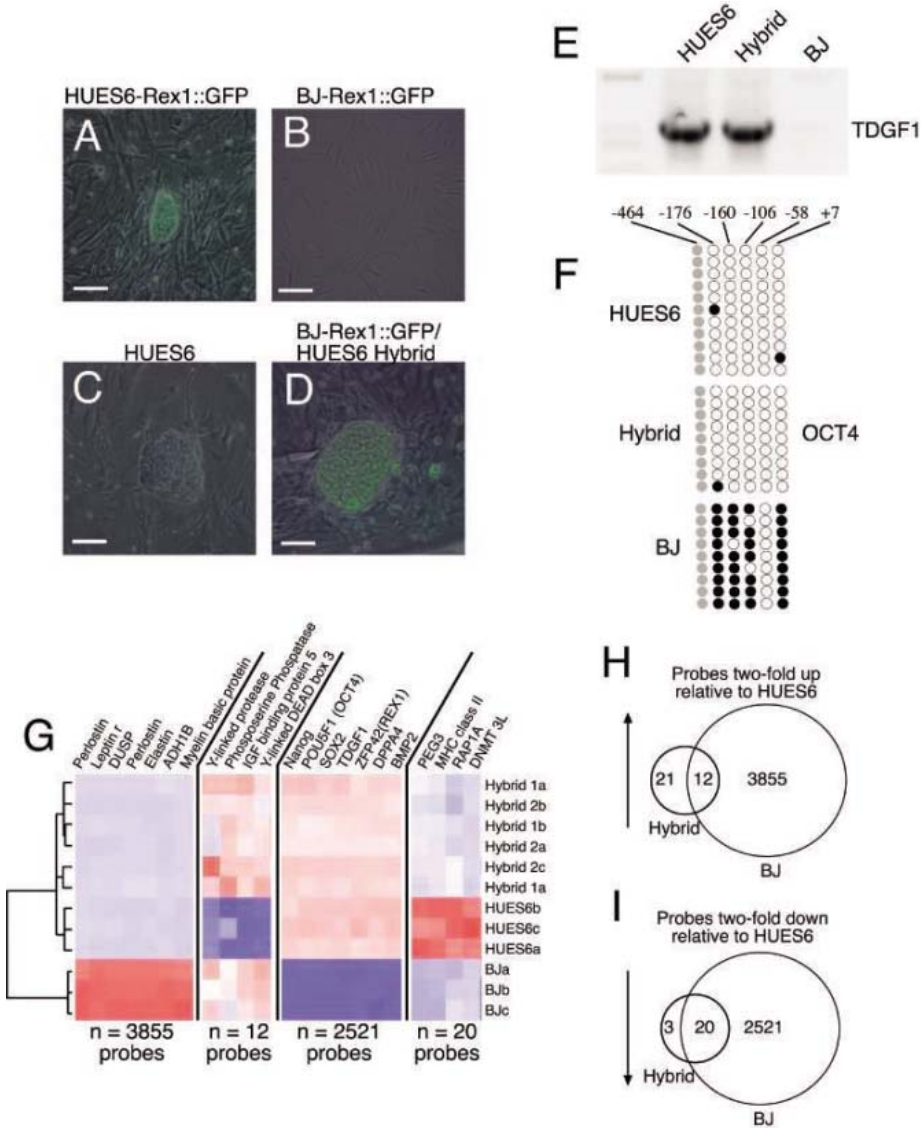

Fig. 3. Transcriptional and epigenetic reprogramming of the somatic genome in hybrid cells. To test for reactivation of embryonic genes from the somatic chromosomes, we used a *Rex1*-GFP reporter that is active when transfected into hES cells (**A**) but silent when transfected into BJ fibroblasts (**B**). When transgenic fibroblasts (B) were fused with nontransgenic HUES6 hES cells (**C**), the resulting cell hybrids expressed GFP (**D**). Scale bars in (A) to (D), 50 μm. To investigate whether transcription of endogenous embryonic genes could be reactivated, we performed reverse transcription PCR with primers specific to the *TDGF1* gene (**E**). Analysis of DNA methylation at the *OCT4* promoter demonstrated that the epigenetic state of the hybrid cells had also been reprogrammed (**F**). To determine whether the transcription of genes specifically expressed in somatic cells was extinguished after cell fusion, we performed genome-wide transcriptional profiling (**G** to **I**). Genes with expression values either twofold higher in BJ cells relative to hES cells (somatic-specific genes) or twofold lower in BJ cells relative to hES cells (hES-specific genes) were noted, and their expression analyzed in the hybrid cells. An Eisengram displaying "heat plots" from representative genes (red, on; blue, off) (G) and Venn diagrams (H and I) depicting the results of pairwise comparisons from transcriptional profiles of the three cell types demonstrate that the somatic program of transcriptional program is silenced in the hybrid cells, whereas the embryonic program predominated.

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Spatial Coordination of Spindle Assembly by Chromosome-Mediated Signaling Gradients

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During cell division, chromosomes are distributed to daughter cells by the mitotic spindle. This system requires spatial cues to reproducibly self-organize. We report that such cues are provided by chromosome-mediated interaction gradients between the small guanosine triphosphatase (GTPase) Ran and importin-β. This produces activity gradients that determine the spatial distribution of microtubule nucleation and stabilization around chromosomes and that are essential for the self-organization of microtubules into a bipolar spindle.

Two models have been proposed to explain how microtubules (MTs) become organized into a bipolar spindle. In the "search-and-capture" model, the dynamic plus ends of MTs nucleated at centrosomes are randomly captured and stabilized at the kinetochores on the chromosomes (*1*). However, in numerous systems, spindle assembly occurs in the absence of extrachromosomal nucleating centers (*2*–*5*). Therefore, another model has been proposed in which chromatin changes the state of the mitotic cytoplasm in its surrounding area and promotes spindle assembly through a self-organization process (*6*, *7*). Experiments have confirmed part of this model. It has been shown that chromatin beads incubated in frog egg extracts promote MT nucleation in their vicinity (*8*) and stabilize MTs at great distances (*9*), which results in the self-organization of a bipolar spindle. Central to this model is the small guanosine triphosphatase (GTPase) Ran, which exists in a guanosine triphosphate (GTP)–bound

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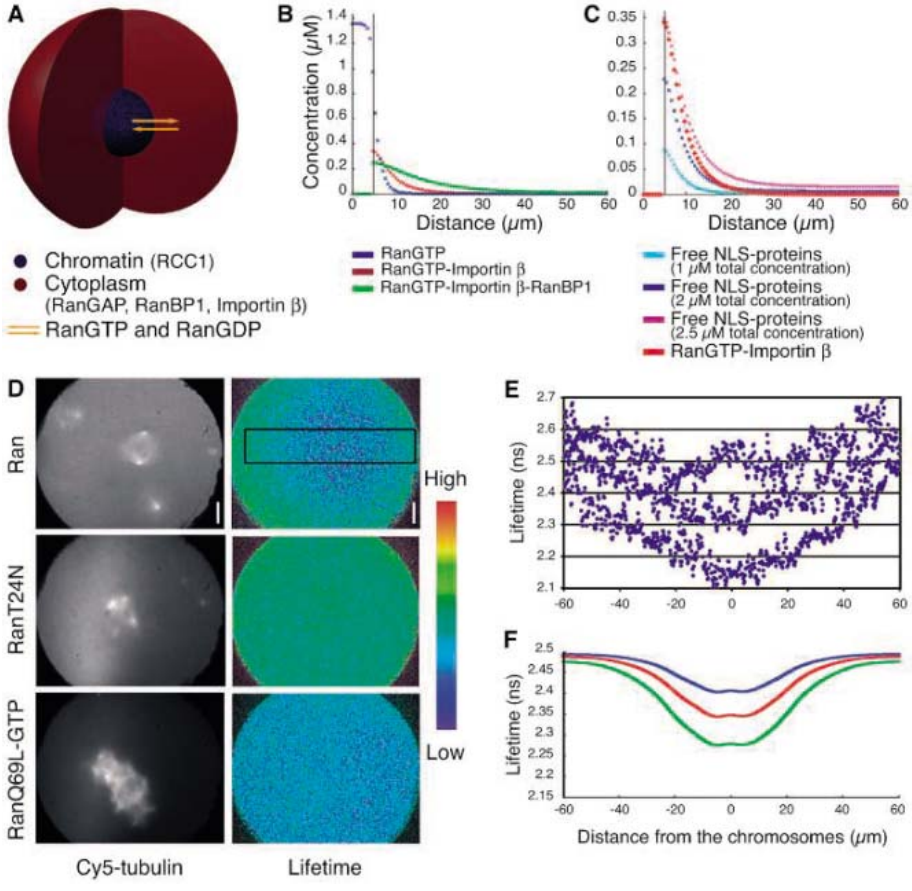

Fig. 1. (**A**) The Ran system was modeled in a spherical space where RanGTP and RanGDP diffuse between chromatin (5 μm radius) and the cytoplasm (60 μm radius), whereas other species are restricted to their own compartments. (**B**) Calculated spatial distribution of RanGTP species concentrations at steady state. Vertical black line, interface between chromatin (left side) and cytoplasm (right side). (**C**) Calculated spatial distribution of free NLS-protein concentration relative to the RanGTP–importin-β distribution. (**D**) Spatial distribution of Alexa 488–Ran lifetime (top right) around a mitotic spindle (top left) (*22*). Blue to green reflects a decreasing interaction between RanGTP and importin-β (color bar). The same experiment, done in the presence of inactive (RanT24N, middle) or active Ran (RanQ69L-GTP, bottom), results in global fluorescence lifetime changes. Scale bars, 15 μm. (**E**) Lifetime profiles were measured around sperm nuclei in different mitotic extracts inside the window (D). (**F**) Theoretical lifetime profiles were computed from relative concentrations of species having a fluorescence lifetime of 2.1 ns or 2.5 ns and ratios of 1:1 (blue), 2:1 (red), and 4:1 (green). Chromatin induces a slight increase in fluorescence lifetime at the center because of the local high Ran-RCC1 complex concentration (2.5-ns fluorescence lifetime). This was observed experimentally to variable extents [top curve in (E)].

active form (RanGTP) close to chromosomes and in a guanosine diphosphate (GDP)-bound inactive form (RanGDP) in the cytoplasm. These two states occur because of the high activity of Ran-GTPase–activating protein (RanGAP), which is cytoplasmic, and a Ran–guanosine nucleotide exchange factor (RanGEF or RCC1) that is localized on chromosomes (*10*–*14*). A short-range RanGTP gradient has indeed been visualized around chromosomes in metaphase *Xenopus* egg extracts and cells (*14*, *15*). However, such a short-range RanGTP gradient cannot explain the observation of long-range effects of chromatin on asymmetric growth of MTs (*9*, *16*). Moreover, free RanGTP does not affect MT nucleation and dynamics directly but rather through the release of MT regulatory NLS-proteins from karyopherins (*13*, *14*). It is thus the resulting spatial distribution of free nuclear localization signal–containing (NLS) proteins that determines where MT nucleation and plus end stabilization occur and that coordinates the spatial organization of MTs into a bipolar spindle.

To examine the formation of RanGTP-dependent downstream effector gradients around metaphase chromosomes, we first modeled the Ran system as a reaction-diffusion process in a spherical space (Fig. 1A). Because of the sequestration of RCC1 on chromosomes and the predominantly homogeneous distribution of RanGAP in the cytoplasm, two different regions harbor different sets of reactions. The central area corresponds to the space occupied by the chromatin that contains RCC1. It is surrounded by the cytoplasm, containing RanGAP and its associated protein RanBP1, as well as the karyopherin importin-β. Ran diffuses in the whole space and forms complexes with RCC1, RanBP1, and importin-β (Fig. 1A) (*17*). The system evolves rapidly and independently of the initial conditions toward a steady state, to generate a steep gradient of free RanGTP around chromosomes (Fig. 1B), which matches the RanGTP gradient measured in *Xenopus* egg extracts (*15*). However, this system also generates RanGTP-dependent long-range gradients that are more physiologically relevant: the RanGTP–importin-β and RanGTP–importin-β–RanBP1 gradients (Fig. 1B). These are the gradients that actually determine both short- and long-range chromatin effects by spatially controlling the release of NLS-proteins that affect MT nucleation and dynamics. Indeed, free NLS-proteins exist only where importin-β is in a complex with RanGTP (Fig. 1C) (*17*). Such long-range gradients exist because the stable RanGTP–importin-β complex prevents RanGAP-induced GTP hydrolysis and thus diffuses away from chromatin (*18*), until dissociation is induced by RanBP1 and importin α binding (*19*, *20*). The RanGTP–importin-β–RanBP1 gradient extends further and builds on the RanGTP–importin-β gradient just as the latter does on the local production of RanGTP around chromosomes.

We investigated directly the span of the RanGTP–importin-β interaction using fluorescence lifetime imaging microscopy (FLIM) (*21*). To measure the interaction between RanGTP and importin-β, fluorescence resonance energy transfer (FRET) between Alexa 488–tagged Ran and Cy3-labeled importin-β was imaged by the decrease in fluorescence lifetime of the Alexa 488 donor (*22*). Extended gradients of RanGTP–importin-β interaction were observed around assembling spindles in metaphase *Xenopus* egg extracts

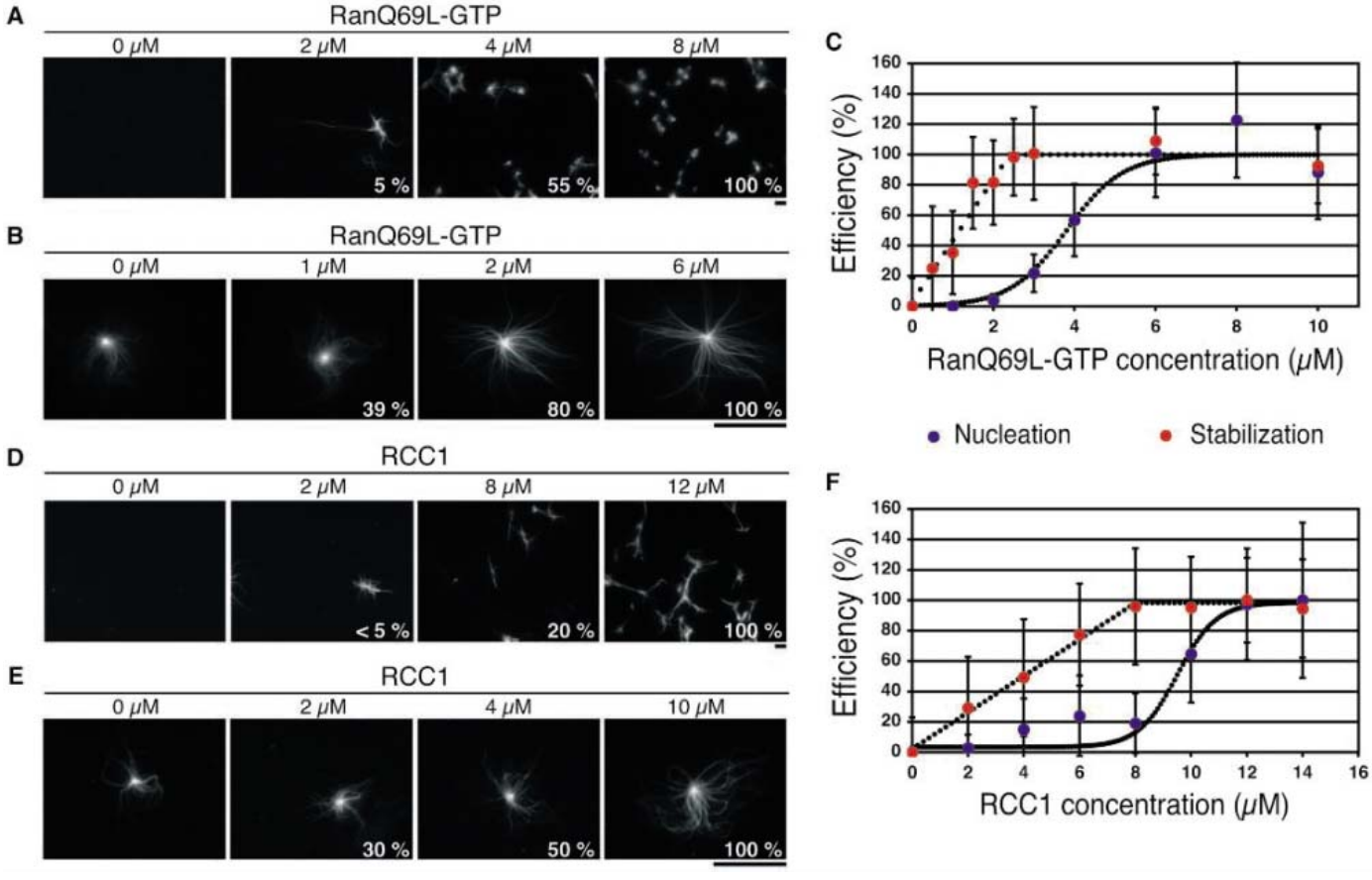

Fig. 2. (**A**) Microtubule nucleation as a function of RanQ69L-GTP concentration. RanQ69L-GTP was added to metaphase extracts containing rhodamine-labeled tubulin (*22*). (**B**) Microtubule stabilization as a function of RanQ69L-GTP concentration. Centrosomes and rhodamine-labeled tubulin were added to extracts together with antibodies against TPX2. (**C**) Microtubule nucleation and stabilization efficiency as a function of RanQ69L-GTP concentration. (**D**) Microtubule nucleation as a function of RCC1 concentration. (**E**) Microtubule stabilization as a function of RCC1 concentration. (**F**) Efficiency of microtubule nucleation and stabilization in response to increasing RCC1 concentrations. Error bars, SD. Scale bars, 10 μm.

(Fig. 1, D and E). The range of a computed spatial lifetime distribution evaluated from the reaction-diffusion theory corresponded well to those reproducibly measured by experiments (Fig. 1, E and F) (*17*). The amplitude variation of their depth (Fig. 1E) is due to the formation of complexes between fluorescent RanGTP and dark endogenous importin-β or other proteins in different experiments, which decreases the dynamic range of the FRET signal.

Because MT nucleation and plus end stabilization occur at different distances from chromosomes (*8*, *9*), such processes could be differentially regulated by the long-range gradient. We therefore quantified MT nucleation and plus end stabilization as a function of global RanGTP concentrations in metaphase *Xenopus* egg extracts and correlated these results with the span of the RanGTP–importin-β complex concentration gradient around chromosomes. RCC1 and a nonhydrolyzable mutant form of RanGTP (in which Gln^{69} is replaced by Leu), RanQ69L-GTP, induce MT nucleation and formation of spontaneous asters in metaphase egg extracts (*12*). We used this assay to quantify MT nucleation in the presence of increasing concentrations of either RCC1 or RanQ69L-GTP (Fig. 2, A and D) (*22*). Half-maximum nucleation efficiency was reached at 4 μM RanQ69L-GTP or 9 μM RCC1 (Fig. 2, C and F). MT nucleation responded in an ultrasensitive manner to the concentration of RanQ69L-GTP and RCC1. To examine the effect of RanGTP concentrations on MT plus end stabilization exclusively, we eliminated the RanGTP-dependent nucleation of MTs by inactivating TPX2 (*23*) and produced asters by adding purified centrosomes that do not require TPX2 to nucleate MTs (Fig. 2, B and E). MT plus end stabilization increased linearly with RanQ69L-GTP or RCC1 concentration up to saturation, reaching half-maximum stabilization efficiency at 1.5 μM RanQ69L-GTP and 4 μM RCC1 (Fig. 2, C and F).

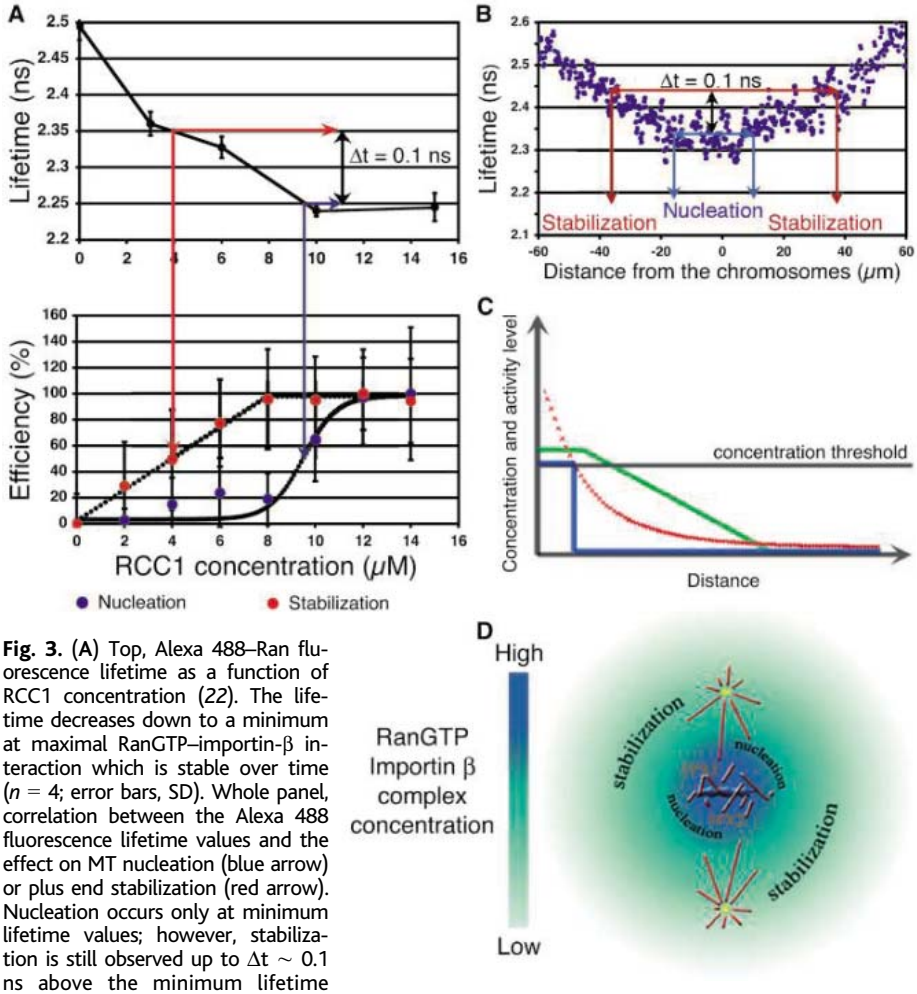

Fig. 3. (**A**) Top, Alexa 488–Ran fluorescence lifetime as a function of RCC1 concentration (*22*). The lifetime decreases down to a minimum at maximal RanGTP–importin-β interaction which is stable over time (n = 4; error bars, SD). Whole panel, correlation between the Alexa 488 fluorescence lifetime values and the effect on MT nucleation (blue arrow) or plus end stabilization (red arrow). Nucleation occurs only at minimum lifetime values; however, stabilization is still observed up to Δt ~ 0.1 ns above the minimum lifetime value. (**B**) Determination of the microtubule nucleation (blue arrows) and stabilization (red arrows) regions around sperm nuclei using an experimental gradient profile (blue dotted curve). (**C**) Differential spatial effects of the RanGTP–importin-β concentration gradient (crossed red line) on microtubule nucleation (blue) and stabilization (green). (**D**) Top view of the effect of the RanGTP–importin-β concentration gradient on microtubule nucleation via TPX2 and plus end stabilization of astral microtubules. Microtubules, red; chromosomes, blue; centrosomes, yellow.

Thus, MT nucleation and plus end stabilization occur at significantly different concentrations of RanGTP, which suggests different spatial regulations of these processes around chromosomes. Using the gradient profiles measured by FLIM around sperm nuclei (Fig. 1E), we could then evaluate the distances at which the gradient of RanGTP–importin-β could affect MT nucleation and plus end stabilization around chromatin (Fig. 3) (*22*). Nucleation should occur in a small region of about 5 ± 5 μm, whereas centrosomal MT plus end stabilization could occur as far as 35 ± 10 μm from chromatin. MT nucleation is restricted to a defined area around chromosomes because of its ultrasensitive response to RanGTP concentration (Fig. 3, C and D), whereas MT plus end stabilization extends over long distances because of its linear response to RanGTP concentration (Fig. 3, A and C).

We next examined whether the formation of a proper bipolar spindle around chromosomes requires the RanGTP–importin-β spatial information gradient. We first added sperm nuclei that contain centrosomes to extracts in which chromosomal MT nucleation had been inhibited by inactivating TPX2 (*22*). This resulted in the formation of aberrant bipolar spindles (*24*, *25*) (Fig. 4A). However, in both control and TPX2-inactivated extracts, 90% of sperm nuclei were connected to centrosomal asters. This shows that chromosomal MT nucleation is not necessary for asymmetric growth of astral MTs and chromosomal capture, but is required for the formation of functional spindles (Fig. 4, B and D). We could now investigate whether a long-range gradient of RanGTP–importin-β was required for the asymmetric growth of MTs toward chromosomes. By varying the concentration of RanGAP and RanBP1 in the extract, we could modulate the reach of the gradient (Fig. 4E) (*17*) and observe the effect on the directional growth of MTs. Decreasing the reach of the gradient by increasing RanGAP and RanBP1 concentrations led to a loss of astral MT asymmetry (Fig. 4A) that was correlated with a strong decrease in the percentage of chromosomes connected to asters (Fig. 4D). Because capture is lost under such conditions, this indicates that a long-range stabilization gradient facilitates biased MT growth and thereby MT capture by chromosomes. A recent theoretical study actually suggests that an unbiased search-and-capture process is unlikely to allow MT capture by chromosomes in a biologically relevant time scale (*26*). However, theory predicts that, in this system, increasing RanGAP and RanBP1 also decreases the amplitude of the RanGTP–importin-β gradient (Fig. 4E) (*17*). This could affect chromosomal capture of MT plus ends simply by lowering the concentration of free NLS-proteins. Therefore, we investigated whether increasing the concentration of free NLS-

proteins globally in the cytoplasm is sufficient to form a spindle. This was done by increasing RCC1 concentration in the extract, which flattened the gradient (Fig. 4E) (*17*). This resulted in a disorientation of MT growth relative to chromosomes, a loss of bipolar spindle assembly, the formation of random MT structures (Fig. 4C) (*17*), and a strong decrease in MT-chromosome connections (Fig. 4D) (*12*). These experiments show that concentration gradients of active MT regulators must operate within well-defined dimensions to achieve proper spatial coordination of spindle assembly (Fig. 3, C and D).

This study shows that chromosomes create a local perturbation in the homogeneous mitotic cytoplasm that generates a radial symmetry. Further breakdown of this radial symmetry by the self-organization of microtubules and their motors (*27*) results in the establishment of a bilateral symmetry centered on chromosomes. Thus, the Ran system does more than simply signal where the chromosomes are; by interfering with the symmetry properties of the mitotic cytoplasm, it functions as a control element that spatially coordinates the self-organization of the MT-chromosome system.

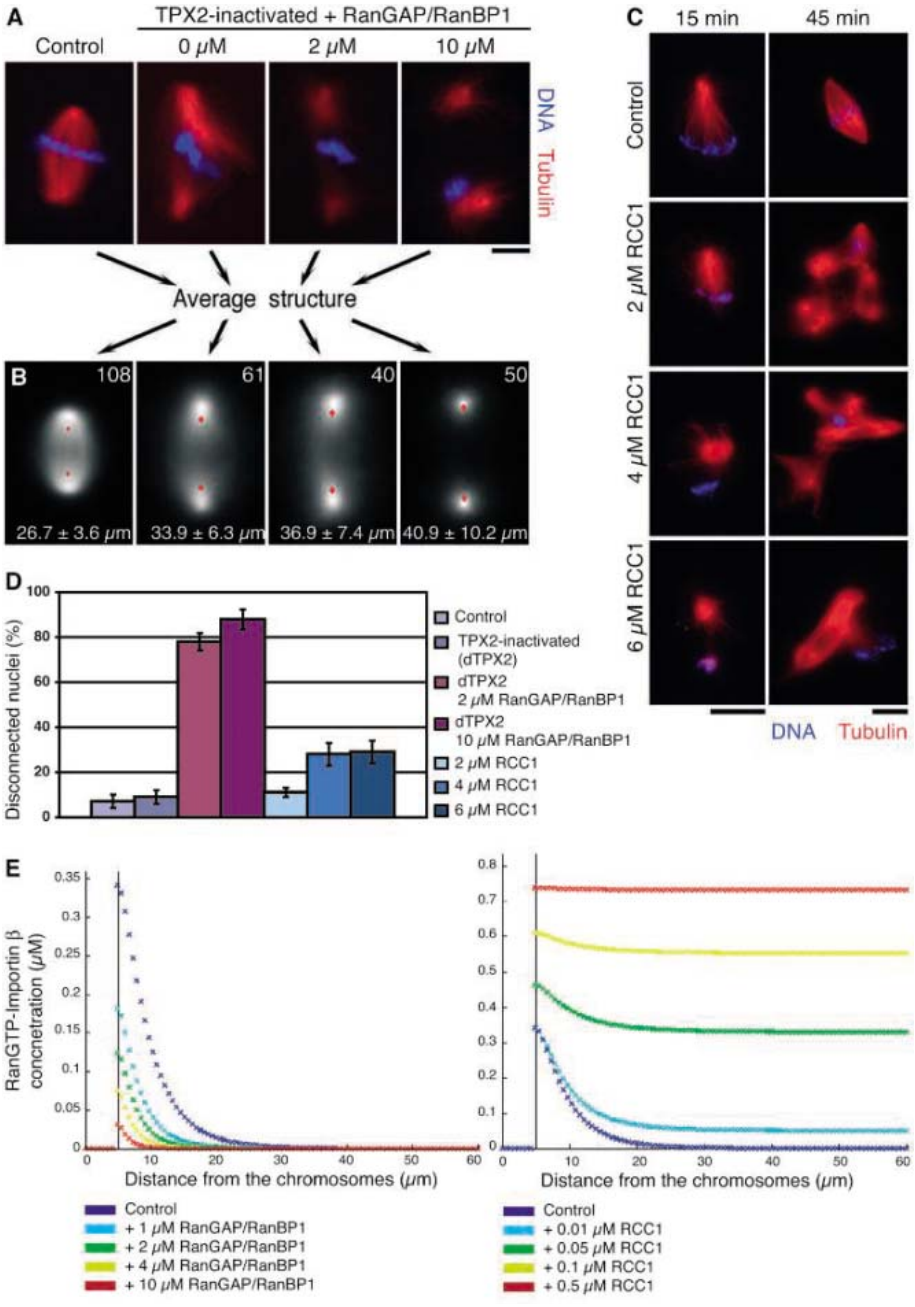

Fig. 4. (**A**) Spindle assembly around sperm nuclei added to M-phase extracts form bipolar spindles (control lane), except in the presence of TPX2 antibody (*22*), and further addition of RanGAP and RanBP1. Scale bar, 10 μm. (**B**) Average microtubule density calculated out of the structures acquired in (A). The asymmetry of centrosomal asters is lost when the microtubule density center of mass (red dots) colocalizes with the centrosomes at the poles of the structures (*21*). Number of structures used and average size ± SD are indicated. (**C**) Perturbation of spindle assembly by increasing RCC1 concentration. Sperm nuclei containing centrosomes added to M-phase extracts produce asymmetric asters after 15 min (*22*). This asymmetry is progressively lost with increasing RCC1 concentrations. After 45 min, sperm nuclei form bipolar spindles (control lane) except in samples containing increasing amounts of RCC1, where disorganized structures are observed. Scale bars, 15 μm. (**D**) Percentage of chromosomes disconnected from centrosomal asters in the samples observed in (A) and in (C) (*22*). Error bars, SD. (**E**) Calculated spatial distribution of RanGTP–importin-β concentration in the presence of increasing amounts of RanGAP and RanBP1 (left) or RCC1 (right). Vertical black line, interface between chromatin (left side) and cytoplasm (right side).

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Supporting Online Material

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Nitrogenase Complexes: Multiple Docking Sites for a Nucleotide Switch Protein

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Adenosine triphosphate (ATP) hydrolysis in the nitrogenase complex controls the cycle of association and dissociation between the electron donor adenosine triphosphatase (ATPase) (Fe-protein) and its target catalytic protein (MoFe-protein), driving the reduction of dinitrogen into ammonia. Crystal structures in different nucleotide states have been determined that identify conformational changes in the nitrogenase complex during ATP turnover. These structures reveal distinct and mutually exclusive interaction sites on the MoFe-protein surface that are selectively populated, depending on the Fe-protein nucleotide state. A consequence of these different docking geometries is that the distance between redox cofactors, a critical determinant of the intermolecular electron transfer rate, is coupled to the nucleotide state. More generally, stabilization of distinct docking geometries by different nucleotide states, as seen for nitrogenase, could enable nucleotide hydrolysis to drive the relative motion of protein partners in molecular motors and other systems.

The ability of nucleotide-hydrolyzing proteins (NTPases) to adopt multiple protein conformations is central to their participation in such biological processes as signal transduction, protein synthesis, membrane transport, muscle contraction, and redox catalysis. Nucleotide-dependent conformational switching in cellular signal transduction pathways, for example, enables guanine nucleotide–binding proteins (G proteins) to selectively bind target proteins and effectors in a nucleotide-state specific fashion (*1*, *2*), which provides a mechanism to temporally regulate downstream processes. In the case of molecular motors, such conformational changes control a progressive sequence of interactions between an ATPase and a macromolecular track, ultimately coupling nucleotide hydrolysis to directional motion (*3*–*5*). To provide a perspective for how these protein-protein and protein-nucleotide interactions evolve during the nucleotide hydrolysis cycle, detailed structural knowledge of the various NTPase-target complexes is essential. In this communication, we report crystal structures for the ATPase-target complexes of nitrogenase in three different states, corresponding to the magnesium adenosine diphosphate (MgADP), MgAMPPCP (a MgATP analog), and nucleotide-free forms. It is noteworthy that different docking geometries are obtained for each of these states, with the distinct interaction sites distributed across a large, contiguous surface of the target protein.

For nitrogenase [reviewed in (*6*–*9*)], ATP hydrolysis controls the timing of the association/dissociation cycle between an electron donor ATPase [iron (Fe-) protein, designated Av2 in *Azotobacter vinelandii*] and a catalytic protein [molybdenum-iron (MoFe-) protein, or Av1]. The coupling stoichiometry appears to be two ATPs hydrolyzed per electron transferred from the Fe-protein to the MoFe-protein. Because the reduction of dinitrogen to ammonia is a six-electron process, multiple cycles of protein complex formation, ATP hydrolysis, and electron transfer are required for substrate reduction (*10*, *11*). MoFe-protein, an $\alpha_2\beta_2$-heterotetramer, contains two unique iron-sulfur clusters: The iron-molybdenum (FeMo-) cofactor, located in the α subunit, provides the site for substrate reduction; the P-cluster bridges the α and β subunits and likely mediates electron transfer between the Fe-protein and FeMo-cofactor. The homodimeric Fe-protein is the electron shuttle for MoFe-protein and contains a [4Fe:4S] cluster coordinated between the two γ subunits. Each γ subunit comprises a predominantly parallel β sheet core flanked by α helices—a motif commonly observed in NTPases—and includes the Walker A and B sequences (*12*) associated with nucleotide binding.

Two observations underscore the pivotal role of highly specific MoFe-protein–Fe-protein interactions in nitrogenase catalysis: First, ATP is hydrolyzed by Fe-protein only in the presence of MoFe-protein. Second, Fe-protein, despite its modestly low reduction potential, is the only known electron donor to effect substrate reduction by MoFe-protein. To elucidate how ATP binding and hydrolysis are coupled to protein-protein interactions and electron transfer, we set out to characterize the nitrogenase complex at different stages of ATP hydrolysis. Because nitrogenase proteins are only transiently associated [$K_d \approx 0.1$ μM (*11*)] during the catalytic cycle, structural characterization of such complexes has been limited to a few artificially stabilized cases. As examples, we previously determined structures of stable Fe-protein–MoFe-protein complexes generated using the putative transition state analog ADP•AlF_4^- [alf-Av2:Av1 (*13*–*15*)]; an inactive, site-directed mutant protein [ΔLeu-γ127–Av2 (*16*, *17*)]; or through chemical crosslinking (*18*, *19*). Of these, the most illuminating was the structure of the alf-Av2:Av1 complex, which indicated a considerable reorientation of the Fe-protein γ subunits during complex formation and nucleotide hydrolysis, whereas the MoFe-protein showed only small changes limited to the protein-protein interface. Notably, rearrangements in alf-Av2 enable two residues on each subunit (Lys-γ10 and Asp-γ129) to extend across the dimer interface to participate in the hydrolysis of the bound nucleotide in the adjacent subunit.

To capture complexes more directly relevant to enzymatic turnover, we cocrystallized Av1 and Av2 under conditions approximating physiological MoFe-protein concentrations [~6 mg/ml (*20*)] and ionic strength (150 to 200 mM), with no nucleotide, MgADP, or the non-hydrolyzable MgATP analog, MgAMPPCP (*21*). Structures of three resulting complexes are described here: the nucleotide-free (nf-Av2:Av1), the MgADP (adp-Av2:Av1), and the MgAMPPCP (pcp-Av2:Av1) complexes at resolutions of 2.1, 3.1, and 2.3 Å, respectively (see table S1 for data collection and refinement statistics). The asymmetric units of nf-Av2:Av1 crystals contain a 1:1 complex; pcp-Av2:Av1 crystals reveal one Av1 tetramer and two, essentially equivalent, Av2 molecules; and adp-Av2:Av1 crystals have two Av1 tetramers, each with two bound Av2 dimers that are less well ordered than in the other complexes (see table S2). As detailed in table S3, nf-Av2 and pcp-Av2 most closely resemble the wild-type Av2 (*22*, *23*) and ΔLeu-γ127–Av2+ATP (*17*) structures, respectively. In the adp-Av2:Av1 structure, three of the four adp-Av2 molecules are most similar to the ADP-bound conformation of free Av2 (*24*); the fourth adp-Av2 most closely resembles free native Av2 (*22*).

The most striking feature of these Fe-protein–MoFe-protein complexes is that the Fe-protein molecules can occupy different interaction sites on the MoFe-protein (Fig. 1). Three separated docking areas may be identified, all on the surface of the MoFe-protein surrounding the two-fold axis approximately relating pairs of α and β subunits. These sites are overlapping and mutually exclusive, and are distinguished by the nucleotide state of the associated Fe-protein. Although each Fe-protein makes at least limited contact with both the α and β subunits, nf-Av2 and the family of four adp-Av2 molecules are positioned at the two

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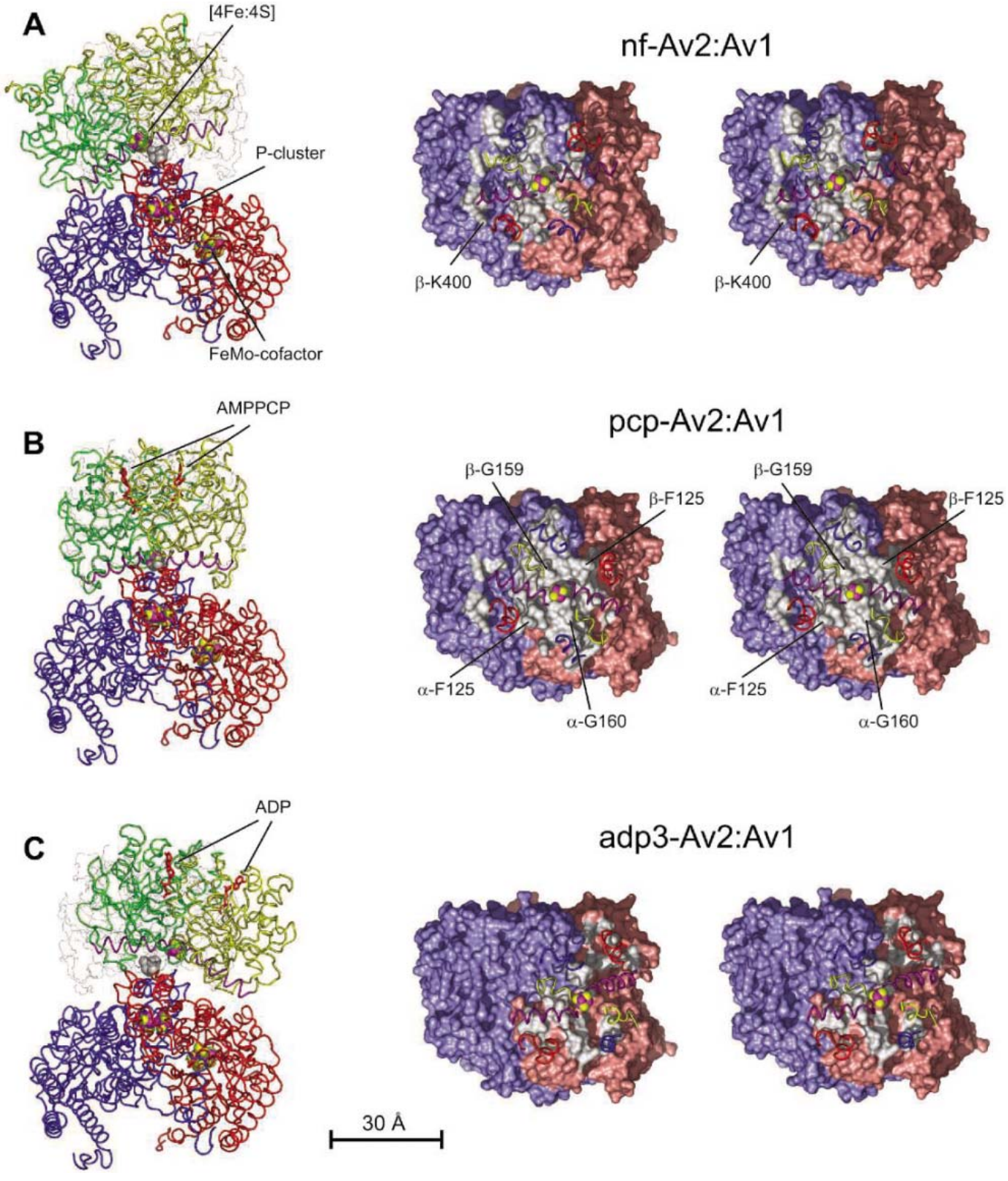

Fig. 1. Protein-protein docking geometries and interaction surfaces in (**A**) nucleotide-free (nf-) Av2:Av1, (**B**) MgAMPPCP (pcp-) Av2:Av1, and (**C**) MgADP (adp-) Av2:Av1 complexes. Here, only one αβ-dimer, encompassing one functional active site, of the Av1 tetramer is depicted; the second αβ-dimer is omitted for clarity. The adp3-Av2:Av1 complex is illustrated by subunit chains KLOP of PDB file 2AFI; figures for the other three adp-Av2 complexes in the asymmetric unit are provided in the Supporting Online Material. Secondary structure representations in the left column show the docking geometry of Av2 on the αβ-dimer surface of Av1 in relation to that of alf-Av2 (gray). Individual γ subunits of Av2 are colored green and yellow, and α and β subunits are shown in red and blue, respectively. γ-100s helices of Av2 molecules are highlighted in magenta to emphasize changes in the flatness of the Av2 docking surface in different nucleotide states. The interaction footprint of Av2 on the surface of Av1 is shown in stereoview and highlighted in white. The interactions of Av2 with Av1 are dominated by the [4Fe:4S] cluster (yellow and magenta spheres), γ-60s loops (red), γ-100s helices (magenta), γ-140s loops (yellow), and γ-170s helices (dark blue).

extremes of the binding surface; nf-Av2 is predominantly bound on the β subunit; the four adp-Av2 conformers interact mostly with the α subunit. In contrast, pcp-Av2 occupies a central position on the binding surface, with its two-fold symmetry axis aligned nearly parallel to the pseudo–two-fold axis relating the α and the β subunits of the MoFe-protein. This docking site is also used in the alf-Av2:Av1 and ΔLeu-γ127–Av2:Av1 complexes (*15*, *17*). As we detect no significant conformational changes in the MoFe-protein between its free form and the ensemble of complex structures now known, the MoFe-protein surface must inherently contain multiple Fe-protein binding sites. In essence, the MoFe-protein surface acts as a rigid template that is explored by the diverse conformations of Fe-protein in various nucleotide states. This behavior is reminiscent of the conformational sampling observed during complex formation between electron transfer proteins (*25*–*28*), as well as protein-protein association reactions more generally (*29*).

If the Fe-protein–MoFe-protein docking geometry is dictated by nucleotide-dependent conformational changes, structural differences in the Fe-protein should be discernible and correlated with the bound nucleotide. In general, Fe-protein conformational changes can be described as a rotational movement of the individual γ subunits about a hinge axis positioned near the [4Fe:4S] cluster [(*17*), Table 1]. These variations modulate the flatness of the Fe-protein interaction surface that is reflected in the relative orientations of the γ-100s helices containing residues γ98 to γ112 of each subunit. The γ-100s helices radiate from the [4Fe:4S] cluster and provide the primary contacts with the MoFe-protein in the different complexes. A measure of the flattening of the Fe-protein surface is provided by the angle ϕ between the axes of these two helices, with a coplanar arrangement defining ϕ = 0°. As shown in Table 1, ϕ varies by ~20° depending on the nucleotide state; the most compact conformation is adopted by alf-Av2 (ϕ = 12°); free Fe-protein structures in the absence (*22*) and presence (*24*) of ADP have ϕ = 34 and 32°, respectively.

The repositioning of the γ subunits required to construct the active site for ATP hydrolysis is coupled to a closing of the Fe-protein dimer interface (decreasing ϕ) and a flattening of the interaction surface with the MoFe-protein. As a consequence, the γ-100s helices in the pcp-Av2 structure can fit snugly in a groove formed by the 120s and the 150s loops of the α and β subunits, placing the [4Fe:4S] cluster within van der Waals distance from the MoFe-protein surface (Fig. 1). The docking site and interactions observed at the interface between component proteins in pcp-Av2:Av1 closely follow those seen in the two previously solved complexes, alf-Av2:Av1 and ΔLeu-γ127–Av2:Av1 (*15*, *17*). This docking geometry allows both γ-60s loops to symmetrically clamp around the protrusions formed by the conserved Phe-α125 and Phe-β125, and enables the γ-140s loops and γ-170s helices to favorably interact with the surface of the ridge formed by the αβ-subunit interface (Fig. 1). The Fe-protein–MoFe-protein interface in the pcp complex features a large network of ionic and hydrogen-bonding interactions involving conserved residues (see table S4 for a list of interprotein H-bonding interactions), likely reflected in the sensitivity of nitrogenase activity to ionic strength (*30*). Of particular note are residues associated with the [4Fe:4S] cluster, ligand Cys-γ97 and Gly-γ133, which participate in main chain–main chain hydrogen bonds with the MoFe-protein. These interactions stabilize the docking interface and may serve to position the cluster for intermolecular electron transfer.

The more open Fe-protein conformations (ϕ ~ 30°) observed in nf-Av2 and the four adp-Av2 molecules cannot simultaneously have both γ-100 helices wedged into the pcp-Av2 docking site on the MoFe-protein. Steric clashes between the MoFe-protein surface and the γ-140s loops and the γ-170s helices of the Fe-protein subunits would further preclude tight binding. Conversely, rigid docking of pcp-Av2 onto the binding sites of the nf-Av2:Av1 or adp-Av2:Av1 complexes indicates that its flat docking surface cannot wrap over the MoFe-protein surface in the same way as these more open Fe-protein conformers. Hence, pcp-Av2 in these alternate docking sites would be expected to form fewer contacts to MoFe-protein. Nevertheless, commensurate with unique Fe-protein conformations, each of the three docking sites has a large buried surface area, as would be expected for extensive surface complementarity between binding partners (Table 1). As adp-Av2 and nf-Av2 exhibit similar overall conformations, factors underlying their selective binding to the homologous α and β subunits, respectively, are less apparent. Intriguingly, the side chains of Glu-γ112 and Lys-β400 are appropriately positioned in the nf-Av2:Av1 complex to form the previously characterized crosslink between these two residues [(*19*, *31*); fig. S2]; although the presence of nucleotide reduces the rate, it does not eliminate formation of the crosslinking, which suggests that both α and β subunit binding sites may be explored by nucleotide bound Fe-protein.

The presence of multiple, nucleotide-dependent protein-protein docking sites has several key implications for nitrogenase and more broadly other nucleotide-dependent systems. First, only a specific subset of the different nucleotide states of the NTPase can form a complex with the target that is catalytically functional, the so-called "on state." For nitrogenase, the "on" state is likely to correspond to the optimal alignment of the redox donor and acceptor for efficient electron transfer. One analytical measure of this alignment is provided by the distance between the Fe-protein [4Fe:4S] cluster and the P-cluster in the three complexes; the ~5 Å closer approach in pcp-Av2:Av1 (as well as the alf-Av2:Av1 and ΔLeu-γ127–Av2:Av1 complexes) than in the nf-Av2:Av1 and adp-Av2:Av1 complexes (Fig. 2 and Table 1) sug-

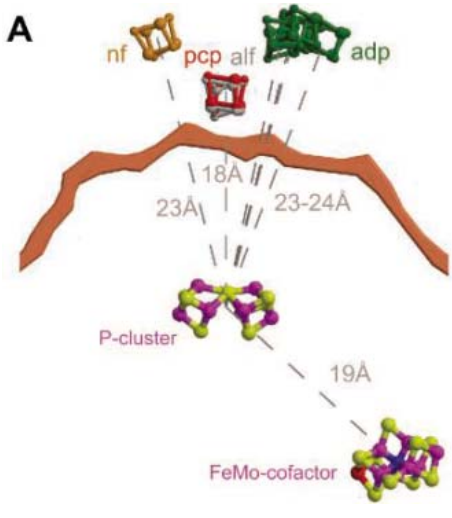

Fig. 2. nf-, pcp-, adp-, and alf-[4Fe:4S]–cluster positions relative to the P-cluster and FeMo-cofactor as viewed perpendicular **(A)** and parallel **(B)** to the local two-fold axis relating an αβ pair of Av1 subunits. The approximate distances shown in the side-view (A) are between the cluster centroids (see also Table 1). The centroid distance between the P-cluster and the FeMo-cofactor is 19.3 Å. The corresponding edge-to-edge [4Fe:4S]–P-cluster distances between the nearest pair of atoms in these structures are ~5 Å shorter. The outline of the Av1 surface is shown in coral.

Table 1. Parameters describing Av2-Av1 docking interactions. Buried surface area denotes the total area on the Av2 and Av1 surfaces buried at the indicated $\alpha\beta\gamma_2$ Av2–Av1 interface, as calculated with the program SURFACE of the CCP4 suite (*33*). ϕ is the angle between the γ-100s helices of each Av2 dimer, with the coplanar arrangement of the helices defining ϕ = 0°, which provides a measure of the flatness of the Av2 docking interface. The [4Fe:4S]–P-cluster distance is defined between the centroids of these clusters in the indicated complex. Entries for adp-Av2 and alf-Av2 are ranges of values for the four independent Av2 dimers in the asymmetric units of adp- and alf-Av2:Av1 (PDB ID: 1M34) complexes, respectively. Individual values for the four adp-conformers are provided in table S5.

Complex	Buried surface area (Å²)	γ-100 helix-helix angle ϕ (°)	[4Fe:4S]–P-cluster distance (Å)
nf-Av2	2800	30	23.2
pcp-Av2	3700	21	17.8
adp-Av2	1600–2000	26–33	22.6–23.7
alf-Av2	3400–3600	12–13	17.5–17.6

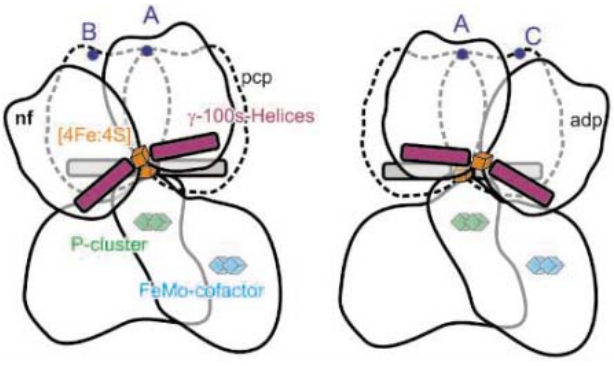

Fig. 3. Schematic representation of Av1-Av2 docking geometry at different nucleotide states. The species representing the pcp-conformer (dotted lines) is included in both cartoons to illustrate the relative docking positions of Av2 molecules. Taking a reference point near the "top" surface of Av2 (away from the interface with Av1) that is positioned on the two-fold axis at a distance of 35 Å from the [4Fe:4S] cluster of each Av2 dimer (point A: pcp-Av2; point B: nf-Av2; point C, adp-Av2), the displacement between pcp-Av2 and nf-Av2 is 19 Å, and that between pcp-Av2 and the four adp-conformers ranges from 10 to 23 Å.

gests an electron transfer rate potentially two to three orders of magnitude faster in the former relative to the latter complexes (*27*, *32*). This effectively renders the nucleotide-free and ADP-Fe-protein forms inactive ("off") for electron transfer. Second, because the docking sites are overlapping, the Fe-protein forms are allosterically competitive with each other, adding a new dimension to the effect of the ADP:ATP ratio in regulation of the downstream target: ATP not only competes with ADP for binding to Fe-protein, but the different nucleotide states of Fe-protein compete for binding to the target MoFe-protein; that is, the ADP-Fe-protein state is not only "off," but it blocks the ATP-Fe-protein "on" state from binding. Third, multiple, connected, and overlapping binding sites with discriminating affinities for different nucleotide state–dependent NTPase conformations are distributed across a single surface of the target protein, which creates the potential for directed motion of the NTPase.

In the nitrogenase complexes, the spatial arrangement of these docking sites could lead to large movements of the Fe-protein during a cycle of nucleotide binding and hydrolysis. For example, in different complexes, as the [4Fe:4S] cluster at the Av1 surface shifts by modest ~7 Å steps (Fig. 3), corresponding residues on the "top" surface of Av2 (away from the interface with Av1) are displaced by up to ~20 Å. Although our studies cannot establish whether directed movement occurs in the nitrogenase system, clearly, the pcp-Av2:Av1 binding site is central with the other two binding sites on the periphery. Nitrogenase and other NTPases utilize nucleotides for their individual physiological purposes; however, these enzymes share common mechanistic elements where nucleotide hydrolysis in a structurally asymmetric protein complex allows the system to evolve through a series of conformational states. Similar principles to those observed in nitrogenase to control electron transfer processes could be used by motor proteins, for example, to achieve long-range, directed motion along a periodic template. This integration of a common chemical reaction, nucleotide hydrolysis, into such functionally diverse contexts is a fascinating aspect to the biological roles of NTPases.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5739/1377/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 to S5
References and Notes

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Toll-Like Receptor 8–Mediated Reversal of CD4$^+$ Regulatory T Cell Function

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CD4$^+$ regulatory T (Treg) cells have a profound ability to suppress host immune responses, yet little is understood about how these cells are regulated. We describe a mechanism linking Toll-like receptor (TLR) 8 signaling to the control of Treg cell function, in which synthetic and natural ligands for human TLR8 can reverse Treg cell function. This effect was independent of dendritic cells but required functional TLR8-MyD88-IRAK4 signaling in Treg cells. Adoptive transfer of TLR8 ligand-stimulated Treg cells into tumor-bearing mice enhanced anti-tumor immunity. These results suggest that TLR8 signaling could play a critical role in controlling immune responses to cancer and other diseases.

Regulatory T (Treg) cells actively suppress host immune responses and, in consequence, play an important role in preventing autoimmunity, as well as in the general regulation of immune responses to tumors and infectious diseases (*1*–*4*). Naturally occurring

$CD4^+$ $CD25^+$ Treg cells represent a subset of Treg cells that exist without specific antigen stimulation and suppress immune responses through a cell-cell contact mechanism (*4*). In comparison, antigen-induced Treg cells mediate immune suppression either through cell-cell contact or through secretion of soluble factors such as interleukin (IL)–10 and transforming growth factor–β (*2*, *4*, *5*). It is possible that both types of Treg cells may have detrimental effects on cancer immunotherapy, because of their potent ability to suppress immune responses (*4*, *5*). Consistent with this hypothesis, increased proportions of $CD4^+$ $CD25^+$ Treg cells have been observed in patients with different types of cancers (*6*, *7*). Furthermore, we recently demonstrated that antigen-specific $CD4^+$ Treg cells present in tumor-infiltrating lymphocyte (TIL) lines suppressed the proliferation of naïve $CD4^+$ T cells through a cell contact–dependent mechanism (*5*, *8*).

Toll-like receptors (TLRs) recognize a set of conserved molecular structures, so called pathogen-associated molecular patterns, that allow them to sense and initiate innate and adaptive immune responses (*9*, *10*). Such responses are generally thought to be produced through the induction of dendritic cell (DC) maturation, which enables DCs to activate naïve T cells and to render effector cells refractory to Treg cells (*11*–*14*). Whether such TLR signals directly regulate the suppressive function of $CD4^+$ Treg cells without the participation of DCs remains unknown.

We established two $CD4^+$ Treg cell lines (Treg102 and Treg164) by pooling a panel of Treg cell clones (*5*, *8*) and tested whether their suppressive function could be reversed. A previous study has shown that stimulation of mouse DCs with TLR ligands, such as lipopolysaccharide (LPS, a TLR4 ligand) and CpG motif–containing oligonucleotides (a TLR9 ligand), induces DCs to secrete cytokines, including IL-6, that render $CD4^+$ effector cells refractory to Treg cell–mediated suppression (*14*). In light of these results, we tested whether DCs might have the potential to influence the suppressive effects of human Treg cells on naïve $CD4^+$ T cell proliferation in the presence of either CpG-A [also called type-D CpG oligonucleotides (*15*–*17*)], LPS, IL-6, or tumor necrosis factor (TNF)–α. Although CpG-A could reverse Treg cell–mediated suppression and restored the proliferation of naïve $CD4^+$ T cells to ~60% of normal levels, LPS, IL-6, and interferon (IFN)–α lacked this ability (Fig. 1A). In control experiments, neither CpG-A nor the cytokines used could appreciably affect the baseline proliferation of any of these cell populations (Fig. 1A and fig. S1). Proliferation experiments performed with plates coated with antibody to CD3 revealed that CpG-A reversed Treg cell–mediated suppression more strongly in the absence of DCs (Fig. 1B). Again, this contrasted with the failure of another class of CpG oligonucleotide, CpG-B (also called type K CpG), as well as of LPS, TNF-α, IL-6, and IFN-α, to block the suppressive activity of $CD4^+$ Treg cells (Fig. 1B). Furthermore, cultured $CD4^+$ Treg cells at 100% purity (*5*, *8*) that had been pretreated with CpG-A for 3 days became nonsuppressive (Fig. 1C). These results demonstrated that CpG-A can directly mediate reversal effect on Treg cell function in the absence of DCs.

Because both CpG-A and CpG-B contain the CpG motif sequences responsible for binding to TLR9, we next tested whether the CpG motif itself conferred the ability to reverse Treg suppressive function. $CD4^+$ Treg cell lines that had been pretreated for 3 days with CpG-A, non-CpG-A (in which the CG sequence was changed to GC), CpG-B, or non-CpG-B (CG changed to GC) (*18*) were used in proliferation assays. Pretreatment with CpG-A and non-CpG-A reversed the suppressive activities of $CD4^+$ Treg cells equally well (Fig. 2A), indicating that the reversal effect of

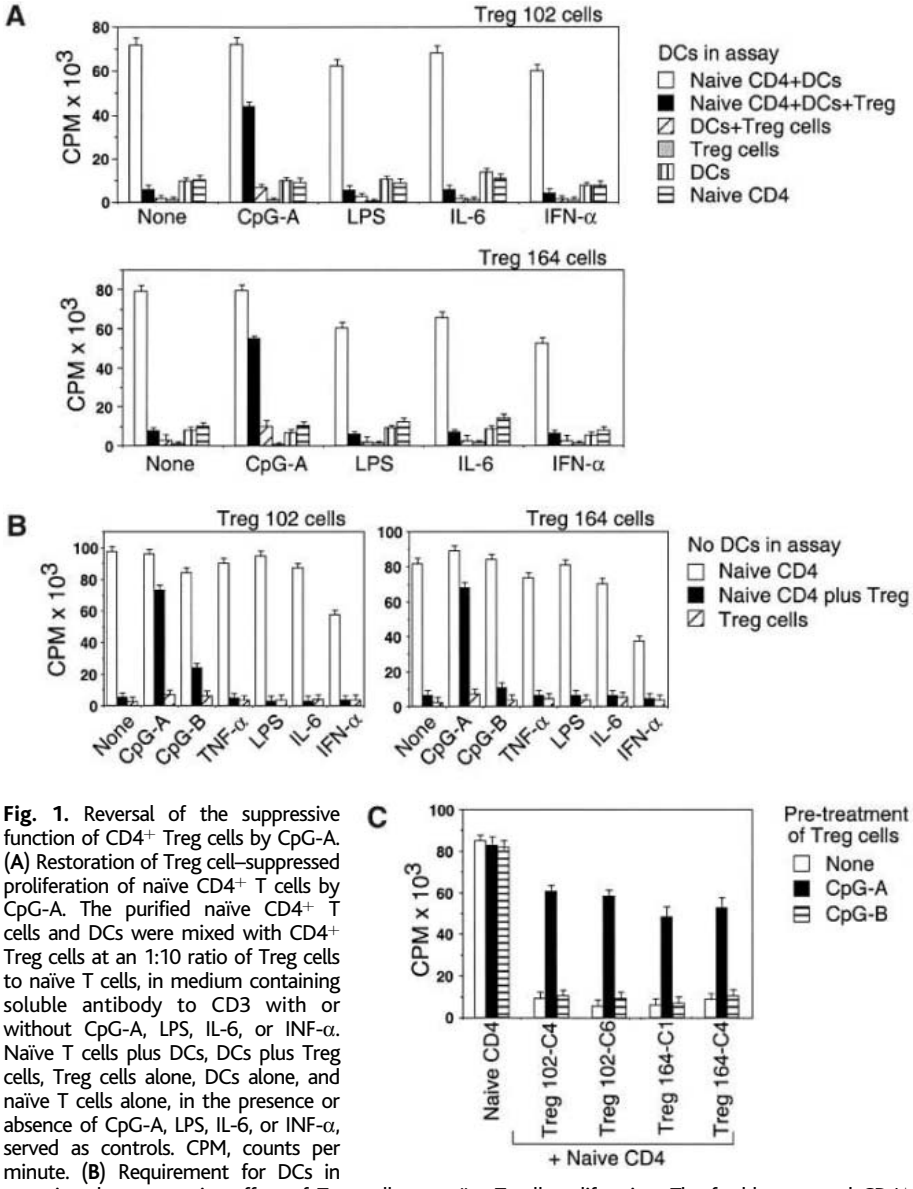

Fig. 1. Reversal of the suppressive function of $CD4^+$ Treg cells by CpG-A. (**A**) Restoration of Treg cell–suppressed proliferation of naïve $CD4^+$ T cells by CpG-A. The purified naïve $CD4^+$ T cells and DCs were mixed with $CD4^+$ Treg cells at an 1:10 ratio of Treg cells to naïve T cells, in medium containing soluble antibody to CD3 with or without CpG-A, LPS, IL-6, or INF-α. Naïve T cells plus DCs, DCs plus Treg cells, Treg cells alone, DCs alone, and naïve T cells alone, in the presence or absence of CpG-A, LPS, IL-6, or INF-α, served as controls. CPM, counts per minute. (**B**) Requirement for DCs in reversing the suppressive effect of Treg cells on naïve T cell proliferation. The freshly prepared $CD4^+$ (responding) T cells were mixed with $CD4^+$ Treg cells in plates coated with antibody to CD3, in medium containing CpG-A, CpG-B, TNF-α, LPS, IL-6, or INF-α but without DCs. (**C**) Reversal of Treg cell suppressive function by pretreatment with CpG-A or non-CpG-A. $CD4^+$ Treg cell clones were pretreated with CpG-A or CpG-B for 3 days before we evaluated their ability to suppress naïve $CD4^+$ T cell proliferation.

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CpG-A did not depend on the CpG motif. To test whether the Poly-G tail (containing five guanosine nucleotides), which is present in CpG-A but absent in CpG-B, was responsible for the observed effect, oligonucleotides were generated in which Poly-G tails had been replaced with Poly-A (CpG-NG). Other oligonucleotides were also tested, in which the CpG motif had been deleted but 10 guanosine nucleosides were retained with the phosphorothioate backbone (Poly-G10) (*18*). The ability to reverse suppression was entirely lost in CpG-NG but retained and enhanced in the CpG-deleted Poly-G10 oligonucleotides, indicating that Poly-G oligonucleotides were necessary and sufficient to reverse Treg cell function, most likely through a receptor distinct from TLR9 (Fig. 2B and fig. S2).

Using $CD4^+$ $CD25^+$ and $CD4^+$ $CD25^-$ T cell populations purified from fresh peripheral blood lymphocytes by fluorescence-activated cell sorting (FACS) (*18*), we again found that the suppressive activity of naturally occurring $CD4^+$ $CD25^+$ Treg cells could be reversed by Poly-G10 oligonucleotides, but not by a control oligonucleotide (Poly-T10) (fig. S3). In culture, proliferation was restricted to naïve $CD4^+$ T cells, whereas Treg cells were nonresponsive, regardless of the presence or absence of Poly-G2 oligonucleotides (Fig. 2C). This T cell receptor–induced proliferation of naïve $CD4^+$ T cells was completely suppressed by both naturally occurring $CD4^+$ $CD25^+$ Treg cells and Treg 102 cells and was reversed by treatment with Poly-G2 oligonucleotides (Fig. 2C). From these experiments, we conclude that ligands capable of reversing Treg cell function can influence both antigen-specific as well as naturally occurring $CD4^+$ $CD25^+$ Treg cells.

Because TLRs have been implicated in the regulation of innate and adaptive immunity and might play a role in controlling Treg cell function (*10*, *12*), we assumed that Poly-G oligonucleotides might mediate reversal of Treg cell function through one of the TLRs. Because MyD88, along with IRAK4, is essential for signaling in TLRs (*10*), we sought to test whether these two adaptors were involved in mediating Poly-G oligonucleotide response. We approached this through specific knockdown using enhanced green fluorescent protein (GFP)–expressing, lentivirus-based RNA interference (*18*, *19*). Inhibition of expression of IRAK4, MyD88, and three of the TLRs by the corresponding small interfering RNAs (siRNAs) was evident in HEK293 cells transfected with target genes (fig. S4). For IRAK4 and MyD88, specific inhibition was also apparent in FACS-sorted GFP^+ (transduced) Treg cells versus untransduced (GFP^-) Treg cells (Fig. 3A). The suppressive ability of these transduced antigen-specific Treg cells, as well as transduced naturally occurring $CD4^+$ $CD25^+$ Treg cells, could no longer be reversed by Poly-G10 oligonucleotides (Fig. 3B and fig. S5), suggesting that direct reversal of the suppressive activity of Treg cells by Poly-G oligonucleotides depends directly on the activity of both adaptors.

Because TLR7, TLR8, and TLR9 have been identified as specific receptors for nucleic acid ligands (*10*, *20*–*25*), we tested whether they may serve as receptors for Poly-G oligonucleotides. Reverse transcription polymerase chain reaction (RT-PCR) (Fig. 3C), as well as quantitative PCR (fig. S6), revealed TLR8 as the only receptor consistently expressed by naturally occurring $CD4^+$ $CD25^+$ Treg cells and antigen-specific Treg cell lines. Consistent with this, the suppressive function of TLR8 siRNA–transduced Treg102 and naturally occurring $CD4^+$ $CD25^+$ Treg cells could not be reversed by Poly-G10, whereas TLR7 or TLR9 siRNA–transduced Treg cells retained their reversible suppressive function (Fig. 3D). Taken together, these results indicate that the TRL8-MyD88 signaling pathway controls the suppressive function of both antigen-specific Treg and naturally occurring $CD4^+$ $CD25^+$ Treg cells.

These results suggest that the TLR8 signaling pathway may be necessary and sufficient for direct reversal of the suppressive function of Treg cells, meaning that it should be possible to reproduce the effect with natural ligands for human TLR8. We found that two such ligands, ssRNA40 and ssRNA33 in complexes with cationic lipid (*23*), completely reversed the suppressive function of antigen-specific Treg102 cells and naturally occurring $CD4^+$ $CD25^+$ Treg cells (Fig. 4A). In contrast, ligands for other TLRs failed to do so (Fig. 4A). Consistent with this result, we found that none of the TLR8 ligands could reverse the suppressive function of Treg102 cells when TLR8 expression was specifically inhibited by siRNA (Fig. 4B).

We next evaluated the ability of Poly-G10 to reverse Treg cell function in a mouse tumor model (*18*, *26*). When injected into B and T cell–deficient $Rag1^{-/-}$ mice, the human

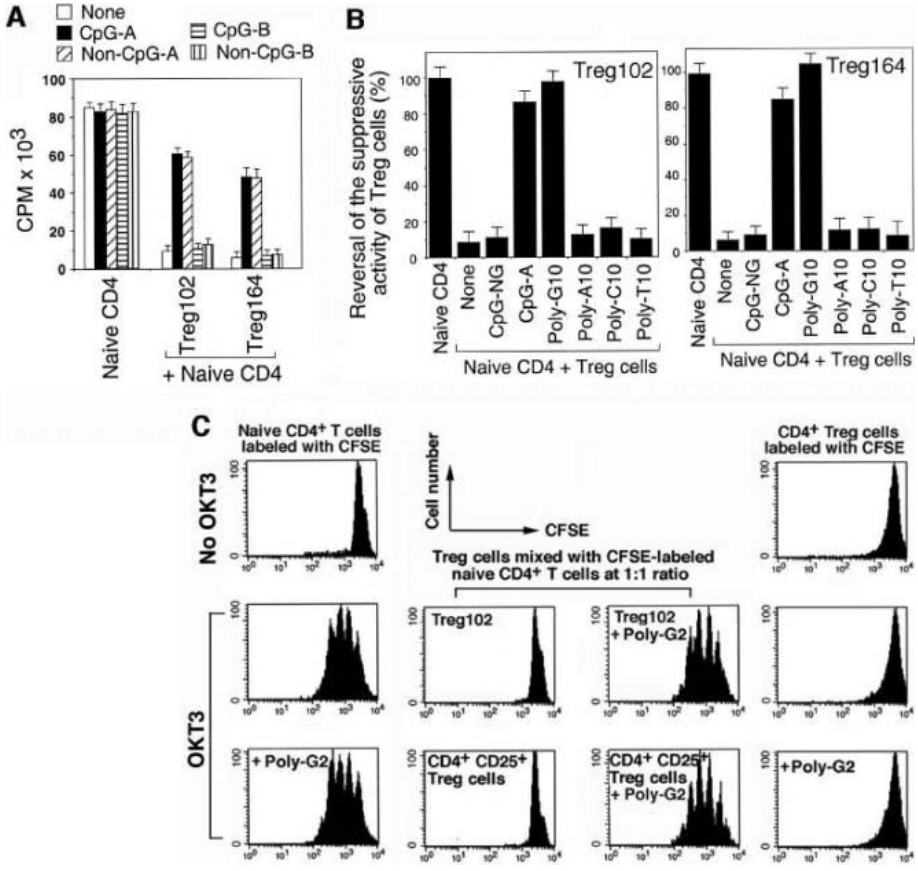

Fig. 2. Identification of sequence elements in CpG-A responsible for the direct reversal of Treg cell suppressive function. (**A**) Reversal of Treg cell function by CpG-A and non-CpG-A. $CD4^+$ Treg cells were pretreated with CpG-A, non-CpG-A, CpG-B, or non-CpG-B for 3 days before we evaluated their ability to suppress naïve $CD4^+$ T cell proliferation. (**B**) Poly-G, but not the CpG motif, is responsible for the observed reversal effect. Naïve $CD4^+$ T cells were mixed with $CD4^+$ Treg cells in the presence of CpG-NG, CpG-A, or Poly-G10. Poly-A10, Poly-T10, and Poly-C10 served as controls for Poly-G10 oligonucleotides. (**C**) Poly-G2 restores the proliferation of naïve T cells suppressed by Treg102 and naturally occurring $CD4^+$ $CD25^+$ Treg cells. Naïve $CD4^+$ T cells or Treg cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE). Cell culture conditions are indicated. After 3 days of culture, cell division was analyzed by FACS gated on the CFSE-labeled cells without OKT3 (antibody to CD3) stimulation.

586mel tumor cell line showed progressive growth but were inhibited when co-injected with autologous, tumor-specific $CD8^+$ TIL586 cells (*26*) (Fig. 4C). When $CD8^+$ TIL586 and Treg102 cells treated with or without Poly-T10 (a control oligonucleotide) were adoptively transferred into tumor-bearing mice, tumor cells again grew progressively and more rapidly than in mice receiving 586mel cells alone ($P = 0.04$), suggesting that the Treg102 cells had inhibited tumor-specific immune responses. By contrast, adoptive transfer of TIL586 cells and Poly-G10–treated Treg102 cells restored, and in some cases enhanced, tumor growth inhibition, compared with that in mice treated with TIL586 cells alone (Fig. 4C) ($P = 0.004$).

To exclude a potential role of the host natural killer cells in controlling tumor growth in vivo, we further investigated the effect of Poly-G treatment on Treg cell-mediated suppression of antitumor immunity in Rag2-γC–deficient mice that lacked B, T, and natural killer cells. The tumor growth rate in Rag2-γC-deficient mice that received TIL586 cells plus Treg cells treated with control oligonucleotides did not differ significantly from that in mice receiving tumor cells without treatment (Fig. 4D) ($P = 0.07$). In contrast, adoptive transfer of TIL586 along with Poly-G10–treated Treg cells into tumor-bearing mice completely restored, but did not enhance, antitumor activity mediated by TIL586 cells ($P = 0.10$). This further supports the conclusion that antitumor immunity mediated by TIL586 cells can be directly controlled by the functional (suppressive versus nonsuppressive) status of Treg cells and that Poly-G oligonucleotide stimulation of Treg cells could reverse this immune suppression.

This study identifies a set of guanosine-containing DNA oligonucleotides and natural TLR ligands that can trigger the TLR8-MyD88-IRAK4 signaling pathway and reverse the suppressive function of different Treg populations. Because most TLRs use the MyD88-IRAK4 pathway, it is not entirely clear why only TLR8 ligands can reverse the suppressive function of Treg cells. In part, this may be explained by the expression pattern of TLRs in Treg cells, because human Treg cells express a relatively high level of TLR8 but little or no TLR7 or TLR9 (Fig. 3C). However, it is also possible that TLR8-MyD88-IRAK4 complexes might recruit a unique downstream signaling pathway of MyD88 required for the control of Treg cell function. Although the findings presented here suggest a mechanism linking TLR8 signaling to the functional regulation of Treg cells, it will be important to establish the physiological relevance of this activity for normal immune responses in vivo. Nevertheless, the results presented here suggest that, by shifting the functional balance between Treg and effector T cells through TLR8 signaling, Poly-G oligonucleotides or similar ligands might be useful in clinical settings to enhance the efficacy of immunotherapy directed toward cancer and infectious diseases.

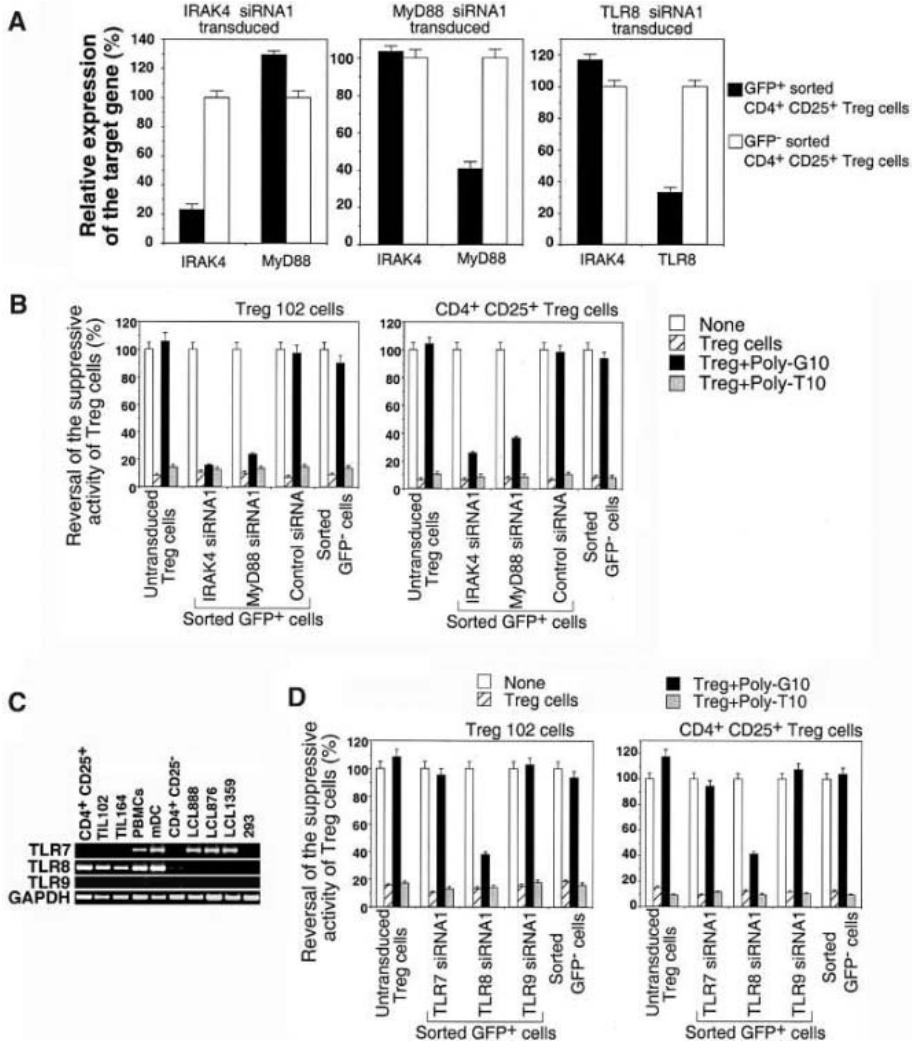

Fig. 3. The TLR8-MyD88-IRAK4 pathway is required to reverse the suppressive function of Treg cells. (**A**) Knockdown of IRAK4, MyD88, and TLR8 in naturally occurring $CD4^+$ $CD25^+$ Treg cells by RNA interference. Specific knockdown of target genes was observed with real-time PCR analysis, and expression of an irrelevant gene was essentially unchanged. (**B**) Evaluation of the reversibility of IRAK4 siRNA, MyD88 siRNA–, or control siRNA–transduced (GFP^+) and untransduced (GFP^-) Treg102 and $CD4^+$ $CD25^+$ Treg cells by Poly-G10 oligonucleotides. Uninfected parental Treg cells and Poly-T10 oligonucleotides served as controls. (**C**) The expression pattern of TLR7, TLR8, and TLR9 in Treg cells as determined by RT-PCR with gene-specific primers. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) amplification was used as an internal control. PBMCs, peripheral blood mononuclear cells; mDC, mature dendritic cell; LCL, EBV-transformed B lymphoblastoid cell line; 293, HEK293 cells. (**D**) The loss of reversible suppressive function by Treg102 and $CD4^+$ $CD25^+$ Treg cells transduced with TLR8 siRNA. Treg cells infected with TLR8 siRNA or TLR9 siRNA served as controls. Treg cells were sorted into transduced (GFP^+) and untransduced (GFP^-) cell populations for testing of their suppressive function in the presence of Poly-G10 or Poly-T10.

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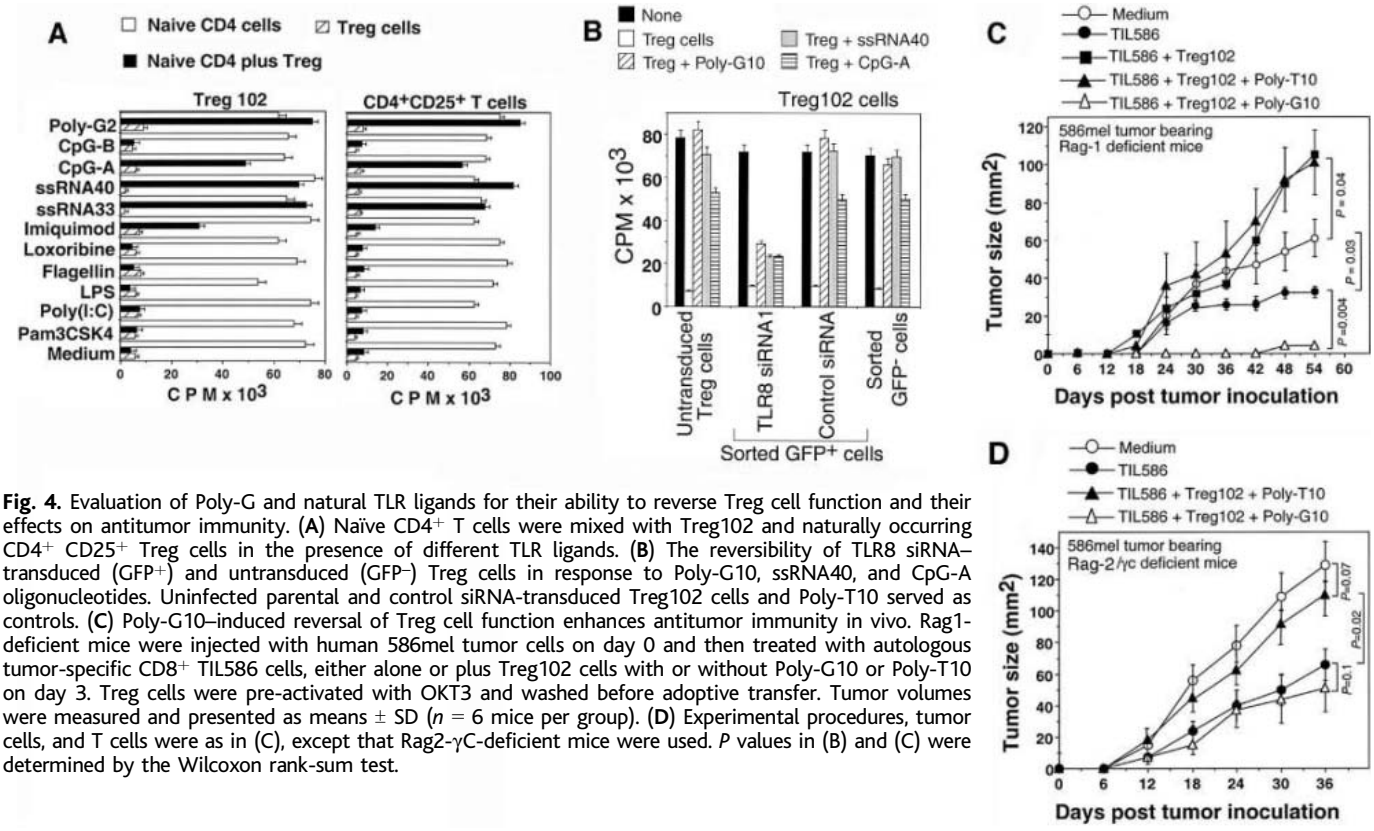

Fig. 4. Evaluation of Poly-G and natural TLR ligands for their ability to reverse Treg cell function and their effects on antitumor immunity. (**A**) Naïve $CD4^+$ T cells were mixed with Treg102 and naturally occurring $CD4^+$ $CD25^+$ Treg cells in the presence of different TLR ligands. (**B**) The reversibility of TLR8 siRNA–transduced (GFP^+) and untransduced (GFP^-) Treg cells in response to Poly-G10, ssRNA40, and CpG-A oligonucleotides. Uninfected parental and control siRNA-transduced Treg102 cells and Poly-T10 served as controls. (**C**) Poly-G10–induced reversal of Treg cell function enhances antitumor immunity in vivo. Rag1-deficient mice were injected with human 586mel tumor cells on day 0 and then treated with autologous tumor-specific $CD8^+$ TIL586 cells, either alone or plus Treg102 cells with or without Poly-G10 or Poly-T10 on day 3. Treg cells were pre-activated with OKT3 and washed before adoptive transfer. Tumor volumes were measured and presented as means ± SD (n = 6 mice per group). (**D**) Experimental procedures, tumor cells, and T cells were as in (C), except that Rag2-γC-deficient mice were used. P values in (B) and (C) were determined by the Wilcoxon rank-sum test.

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Materials and Methods
Figs. S1 to S6
References and Notes

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Molecular Mechanism for Switching of *P. falciparum* Invasion Pathways into Human Erythrocytes

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The malaria parasite, *Plasmodium falciparum*, exploits multiple ligand-receptor interactions, called invasion pathways, to invade the host erythrocyte. Strains of *P. falciparum* vary in their dependency on sialated red cell receptors for invasion. We show that switching from sialic acid–dependent to –independent invasion is reversible and depends on parasite ligand use. Expression of *P. falciparum* reticulocyte–binding like homolog 4 (PfRh4) correlates with sialic acid–independent invasion, and PfRh4 is essential for switching invasion pathways. Differential activation of PfRh4 represents a previously unknown mechanism to switch invasion pathways and provides *P. falciparum* with exquisite adaptability in the face of erythrocyte receptor polymorphisms and host immune responses.

Plasmodium falciparum causes the most lethal form of malaria, a devastating disease responsible for vast morbidity and loss of life. Invasion of erythrocytes by malaria parasites involves complex interactions between multiple ligands and receptors (*1*). Some *P. falciparum* strains predominantly use ligands that bind to sialic acid–containing erythrocyte receptors, and invasion is compromised when red cells are treated with neuraminidase (*2*, *3*). In contrast, other strains use ligands that bind to receptors independently of sialic acid. W2mef parasites have the capacity to switch from sialic acid–dependent to –independent invasion by selection on neuraminidase-treated erythrocytes (*4*–*6*). Consequently, disruption of sialic acid–dependent ligand EBA175 in the strain W2mef (W2mefΔ175) was possible and also resulted in a switch from sialic acid–dependent to –independent invasion (*5*, *6*). The molecular basis of switching to sialic acid–independent invasion has been unknown.

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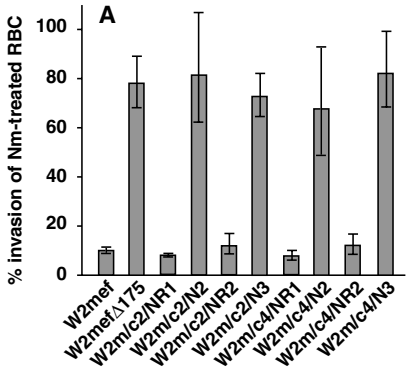
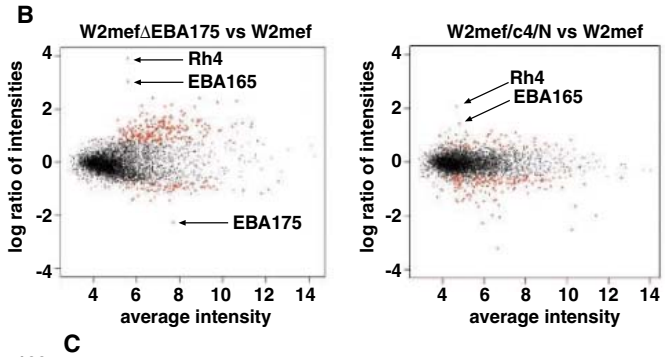




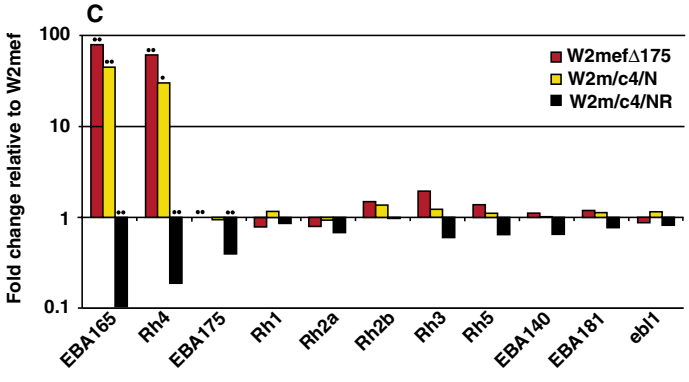
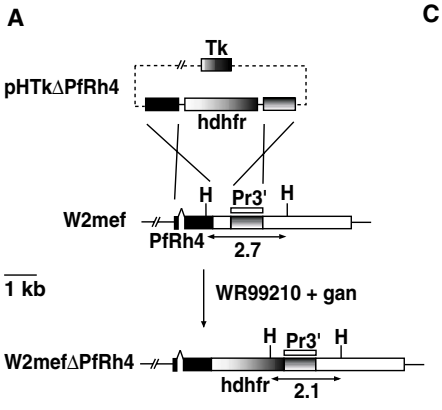
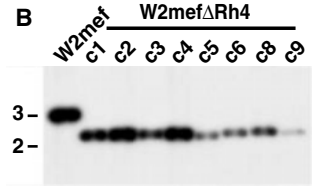


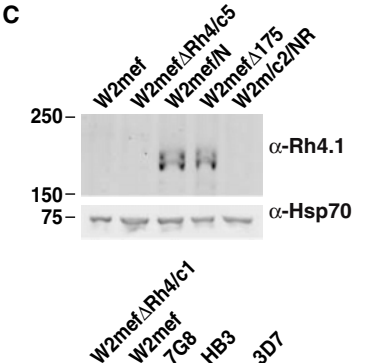
Fig. 1. Sialic acid–dependent and –independent invasion and global transcription in *P. falciparum*. (**A**) Erythrocytes were treated with neuraminidase to remove surface sialic acid residues. The percentage invasion into neuraminidase (Nm)–treated erythrocytes is shown relative to invasion into untreated erythrocytes. W2mefΔ175 has the *EBA175* gene disrupted (*6*). W2mef/c2 and c4 were cloned (fig. S1A) and selected for growth on neuraminidase-treated erythrocytes to obtain W2m/c2/N and W2m/c4/N. After growth on untreated erythrocytes over several months, revertants were derived (W2m/c2/NR1 and W2m/c4/NR1), which were indistinguishable from W2mef. Revertants were reselected on neuraminidase-treated erythrocytes to derive W2m/c2/N2 and W2m/c4/N2. Reversion (W2m/c2/NR2 and W2m/c4/NR2) and reselection (W2m/c2/N3 and W2m/c4/N3) could be reproduced successively in both clones of W2mef. (**B**) Oligonucleotide array analysis comparing transcriptional profiles in W2mef (sialic acid–dependent) with W2mefΔ175 and W2mef/c4/N (both sialic acid–independent). The genes that differ in transcription most relative to sialic acid–dependent W2mef are annotated: *EBA165*, erythrocyte binding antigen 165; *PfRh4*, reticulocyte binding homolog 4; and *EBA175*, which was essentially undetectable in W2mefΔ175. See also fig. S2, A and B. (**C**) Real-time PCR. Gene expression in W2mefΔ175, W2m/c4/N, and W2m/c4/NR is expressed relative to W2mef for genes of the *ebl* and *PfRh* families. $*P < 0.05$, $**P < 0.01$. A transcript for the *EBA175* gene was undetectable in W2mefΔ175. See also fig. S2C.

To address this issue, two clonal lines of W2mef, W2m/c2 and W2m/c4 (fig. S1A), were selected on neuraminidase-treated erythrocytes to select parasites switched to sialic acid–independent invasion to derive W2m/c2/N and W2m/c4/N, both of which showed invasion comparable to that of W2mefΔ175. These parasites were grown on normal erythrocytes for several months to derive W2m/c2/NR1 and W2m/c4/NR1, which had reverted to sialic acid–dependent invasion (Fig. 1A). Both revertant clones were reselected on neuraminidase-treated erythrocytes and invaded using sialic acid–independent receptors. Further rounds of growth on normal erythrocytes and reselection on neuraminidase-treated erythrocytes showed that the ability to switch invasion pathways was reproducible (Fig. 1A). The ability of W2mef to switch from sialic acid–dependent to –independent invasion is reversible and demonstrates the plasticity of *P. falciparum* in the face of selective pressures such as altered erythrocyte receptors.

Microarrays of *P. falciparum* (*7*) were used to reveal any transcriptional switch between W2mef versus W2mefΔ175 and W2m/c4/N (Fig. 1B and fig. S2, A and B). *PfRh4* (PFD1150c) (*8*) and *EBA165* (PFD1155w) were the only genes to show reproducible and significant transcriptional differences. *PfRh4* and *EBA165* occur within the *P. falciparum* genome in a head-to-head orientation on chromosome 4, and it is possible they are coregulated (*9*). The protein encoded by *PfRh4* is homologous to other reticulocyte binding–like







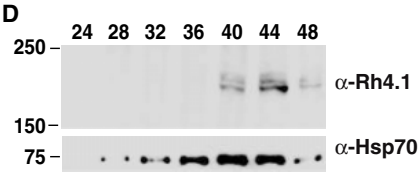


Fig. 2. Disruption of *PfRh4* and expression of PfRh4 protein in *P. falciparum*. (**A**) Schematic of the wild-type and disrupted *PfRh4* loci. The *hDHFR* gene was inserted into *PfRh4* by double crossover recombination. Southern analysis was performed using Hind III (H)–digested DNA with probe Pr3′. (**B**) Southern blot of the *PfRh4* locus in W2mef and clones of W2mefΔRh4 (fig. S1B). Clones 1 to 4 and clones 5 to 9 were derived from two independent transfections. Molecular sizes are shown on the left (in kb). The absence of the 2.7-kb wild-type band and the presence of a 2.1-kb band indicate that the *PfRh4* locus is disrupted in all clones. (**C**) Schizonts were probed with α-Rh4.1 (fig. S3B) (top) or α-Hsp70 (bottom) antibodies. W2mefΔ175 and W2mef/N (sialic acid–independent) express PfRh4, which was absent in W2mef, W2mefΔRh4/c5, W2mefΔRh4/c1, and W2m/c2/NR (sialic acid–dependent) parasites. Molecular sizes are indicated on the left (in kD). The parasite lines 7G8, HB3, and 3D7 also express PfRh4 and invade by sialic acid–independent pathways. (**D**) Expression of PfRh4 over the asexual life cycle is shown for W2mefΔ175, and time points are indicated in hours.

proteins (PfRh), including PfRh1 (PFD0110w) and PfRh2b (MAL13P1.176) that have been implicated in *P. falciparum* invasion of erythrocytes (*10*–*12*), and this family is related to other invasion proteins in *Plasmodium yoelii* (*13*) and *Plasmodium vivax* (*14*). *EBA165* is a member of the erythrocyte binding–like (ebl) family that includes EBA175; however, current data suggest that it is a transcribed pseudogene (*15*). Sequencing of *EBA165* in W2mefΔ175 and W2mef showed that frameshift mutations were present, suggesting that it was unlikely to encode a protein and was only activated by its proximity to *PfRh4*. Additionally, antibodies to putative EBA165 did not bind to the predicted protein in W2mefΔ175 (fig. S3A).

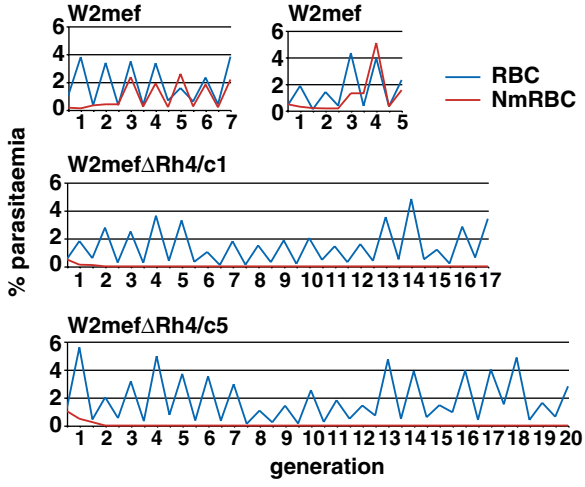


Fig. 3. Disruption of *PfRh4* blocks switching from sialic acid–dependent to –independent invasion of host erythrocytes. Parasites were grown on either untreated or neuraminidase-treated erythrocytes. Starting parasitemia was ~0.5%, and parasites were subcultured every 48 hours to prevent overgrowth. In two independent experiments, W2mef switched invasion pathway from sialic acid dependence to independence by generation 3 (day 6). W2mefΔRh4/c.1 and c.5 (derived from independent transfection experiments) were unable to switch to sialic acid–independent invasion, and no parasites were observed after extended culture on neuraminidase-treated red cells (up to 40 days) or after the culture had been returned to untreated erythrocytes. The same result was observed for all W2mefΔRh4 clones (fig. S3C).

To validate transcriptional changes seen in microarrays, we used real-time polymerase chain reaction (RT-PCR) of sialic acid–dependent parasites W2mef and W2m/c4/NR, and sialic acid–independent parasites W2mefΔ175 and W2m/c4/N (Fig. 1C and fig. S2C). Transcription of *PfRh4* and *EBA165* increased ~60- to 80-fold in the sialic acid–independent lines compared with the sialic acid–dependent lines, confirming the microarray results. In comparison, other members of the *ebl* and *PfRh* families showed relatively minor increases in transcription. These results suggest that activation of the *PfRh4* gene is required for switching from sialic acid–dependent to –independent invasion.

We constructed transgenic parasites in which the *PfRh4* gene was disrupted (W2mefΔRh4/c1-9) and used PfRh4-specific antibodies to determine expression patterns (Fig. 2 and fig. S3B). Attempts to disrupt *PfRh4* in the sialic acid–independent parasite lines 3D7 and 7G8 were unsuccessful; although this does not verify that *PfRh4* is essential in these sialic acid–independent parasites, it does suggest a functionally important role in the invasion of normal erythrocytes. Specific antibodies did not detect PfRh4 in sialic acid–dependent parasites W2mef and W2m/c4/NR, nor in any

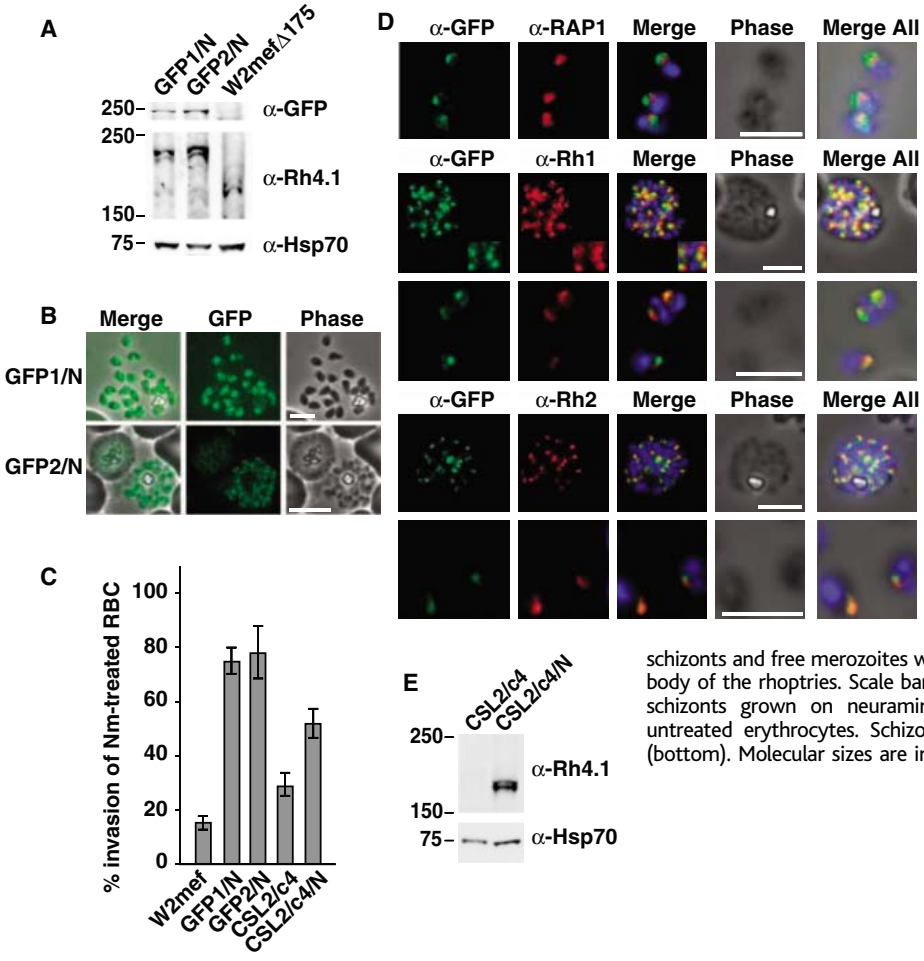


Fig. 4. Subcellular localization of PfRh4 in *P. falciparum* lines with different invasion pathways. (**A**) Two independent clones (fig. S1C), in which PfRh4 is tagged with GFP (see fig. S5, A to C for construction details), were selected for sialic acid–independent invasion by growth on neuraminidase (Nm)–treated erythrocytes. Western analysis of sialic acid–independent W2mef-Rh4GFP/N clones [W2mef-Rh4GFP1/N (GFP1/N) and W2mef-Rh4GFP2/N (GFP2/N)] shows that the PfRh4-GFP chimeric protein was larger than the endogenous form and no GFP-tagged protein was detected in W2mefΔ175. Molecular sizes are indicated on the left (in kD). (**B**) GFP1/N and GFP2/N showed fluorescence, and in many cases it was possible to localize the GFP to the apical tip of merozoites (see also fig. S5D). (**C**) Invasion of W2mef, GFP1/N, GFP2/N, CSL2/c4 and CSL2/c4/N into neuraminidase-treated erythrocytes. The *P. falciparum* strain CSL2 was cloned (fig. S1D) and grown on neuraminidase-treated erythrocytes. (**D**) PfRh4 colocalizes with PfRh1 and PfRh2 in schizonts (also see enlargements, second row of panels) and merozoites. W2mef-Rh4GFP1/N was used to test colocalization with PfRh1, PfRh2, and RAP1. All proteins localize to the apical pole and show pronounced overlap in segmenting schizonts and free merozoites with the exception of RAP-1, which is localized to the body of the rhoptries. Scale bar, 5 μm. (**E**) PfRh4 is expressed more highly in CSL2 schizonts grown on neuraminidase-treated erythrocytes (CSL2/c4/N) than on untreated erythrocytes. Schizonts were probed with α-Rh4.1 (top) or α-Hsp70 (bottom). Molecular sizes are indicated on the left (in kD).

of the W2mefΔRh4 cloned lines (Fig. 2C). In contrast, PfRh4 was expressed in sialic acid–independent lines W2mef/N and W2mefΔ175, as well as in 7G8, HB3, and 3D7. Western analysis was performed during the course of the 48-hour asexual blood stage life cycle of W2mefΔ175 parasites. PfRh4 was detected in mature schizonts (Fig. 2D), concomitant with apical organelle development and expression of other ligands involved in the invasion process (*1*, *16*).

W2mΔRh4 parasites were grown on normal or neuraminidase-treated erythrocytes to determine if they could switch to sialic acid–independent invasion. W2mefΔEBA181 parasites (*17*) were generated in the same way as W2mefΔRh4, and as expected, this line was able to switch to sialic acid–independent invasion (fig. S4A). Although W2mΔRh4 parasites grew normally on untreated erythrocytes (Fig. 3), they were unable to switch to sialic acid–independent invasion even after extended culture on neuraminidase-treated erythrocytes (Fig. 3 and fig. S3C), suggesting that invasion was completely blocked at the first generation. Therefore, transcriptional activation of the *PfRh4* gene and expression of the PfRh4 protein are required for switching of W2mef from sialic acid–dependent to –independent invasion.

We constructed two independent transgenic parasite lines that expressed PfRh4 as a chimeric protein with green fluorescent protein (GFP) to determine if subcellular localization of PfRh4 is consistent with a role in merozoite invasion; the results were identical (figs. S1C and S5). The W2mef-Rh4GFP parasites could switch invasion pathways and invaded neuraminidase-treated erythrocytes efficiently, indicating that activation and function of PfRh4 were preserved (Fig. 4C). The GFP-tagged PfRh4 protein showed the expected increase in molecular weight (Fig. 4A). Segmenting schizonts and merozoites of W2mef-Rh4GFP1N/2N displayed fluorescence apical to the nucleus (Fig. 4B, fig. S5D). PfRh4 colocalized well with PfRh2a/b in segmenting schizonts, and the overlap condensed into a single apical dot in free merozoites in which PfRh2a and b are present in the neck of the rhoptries (*11*, *12*) (Fig. 4D). PfRh4 was more apical than RAP1, a protein located within the body of the rhoptries (*18*). Therefore, PfRh4 is located at the apical tip of free merozoites, consistent with a direct function in invasion of erythrocytes.

We tested several sialic acid–dependent strains for growth on neuraminidase-treated erythrocytes to determine if the ability to switch invasion pathways and use different receptors for invasion is a general property of *P. falciparum*. Cloned lines of CSL2 (fig. S1D) were sialic acid–dependent but adapted to sialic acid–independent invasion in a similar way to W2mef (Fig. 4C) in association with elevated expression of PfRh4 protein (Fig. 4E).

We have shown that activation of sialic acid–independent invasion is regulated by differential gene expression and silencing of *PfRh4*. Activation of *PfRh4* occurs at a low level, and these variant parasites can be selected by growth on erythrocytes lacking sialic acid or by genetic ablation of the *EBA175* gene. Silencing of the active *PfRh4* gene occurs over time when parasites are returned to normal erythrocytes, showing that the switch in invasion pathways can occur in both directions in the presence of functional EBA175. The activation of *PfRh4* in response to loss of EBA175 function suggests that the PfRh and ebl protein families show some overlap with respect to their function in invasion. The ability to switch receptor usage for invasion from sialic acid–dependent to –independent pathways represents a previously unknown strategy to evade host receptor polymorphisms and immune mechanisms and has important implications for the design of vaccines against malaria parasites.

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Computational Improvements Reveal Great Bacterial Diversity and High Metal Toxicity in Soil

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The complexity of soil bacterial communities has thus far confounded effective measurement. However, with improved analytical methods, we show that the abundance distribution and total diversity can be deciphered. Reanalysis of reassociation kinetics for bacterial community DNA from pristine and metal-polluted soils showed that a power law best described the abundance distributions. More than one million distinct genomes occurred in the pristine soil, exceeding previous estimates by two orders of magnitude. Metal pollution reduced diversity more than 99.9%, revealing the highly toxic effect of metal contamination, especially for rare taxa.

For any complex system, the number and relative abundance of parts is fundamental to a quantitative description of the system. Quantification provides a framework to compare equilibrium and dynamic properties and, for biological communities, to evaluate perturbations such as pollution, global climate change, and foreign species encroachment. To quantify plant and animal communities, ecologists survey the number and relative abundance of species (i.e., species-abundance distributions) (*1*, *2*). However, effective measurement of bacterial species-abundance distributions has eluded

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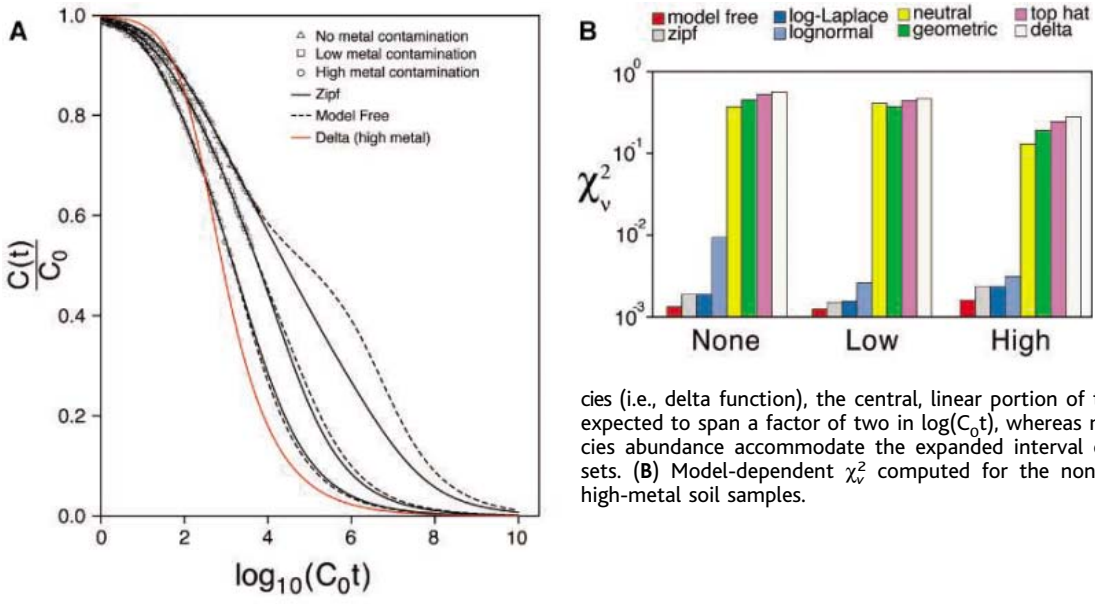

Fig. 1. (**A**) Soil DNA reassociation data with best-fitting Zipf- and model-free–based Cot curves. The best fitting delta function for the high-metal soil is shown for comparison. The samples and the *E. coli* DNA used as a control exhibited equal optical purity (measured as percent hyperchromicity) (*21*), which indicates that the differences in sample diversity were not experimental artifacts arising from DNA impurities. For samples with equi-abundant species (i.e., delta function), the central, linear portion of the DNA reassociation curve is expected to span a factor of two in $\log(C_0t)$, whereas models that allow unequal species abundance accommodate the expanded interval observed in experimental data sets. (**B**) Model-dependent χ^2_ν computed for the noncontaminated, low-metal, and high-metal soil samples.

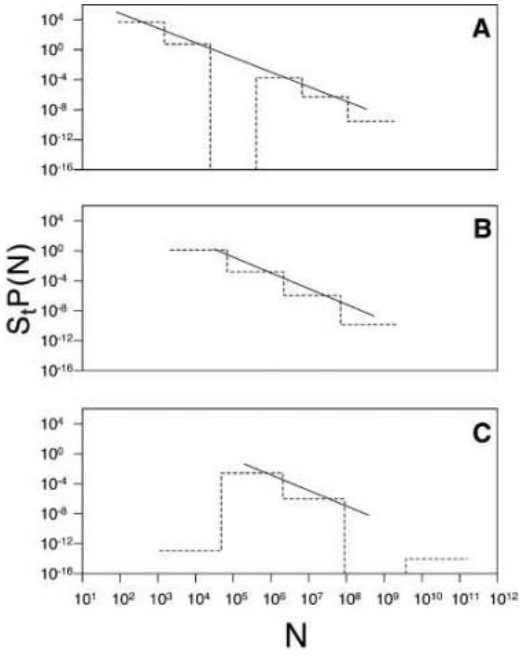

Fig. 2. Zipf (solid line) and model-free (dotted line) species-abundance plots normalized to S_t for (**A**) the noncontaminated data set, (**B**) the low-metal data set, and (**C**) the high-metal data set.

microbiologists owing to the overwhelming complexity of bacterial communities.

Surveys of bacterial communities are typically attempted by counting small subunit rRNA (16S rRNA) gene sequences. Aside from the technical difficulties and biases (*3*), the survey size required for accurate analysis of soil communities is impractically large. Accurately estimating diversity in a community with a log-normal species-abundance distribution requires sampling about 80% of the species (*4*, *5*). For a typical gram of soil containing a billion bacterial cells, a survey of at least 10^6 16S rRNA gene sequences, three orders of magnitude larger than current survey efforts, would be required to sample 80% of diversity in a community with 10,000 species.

Measuring total genetic diversity overcomes the limitations of surveys (*6*). By using two simplifying assumptions, genetic diversity can be translated into species diversity. Genetic diversity can be inferred from DNA reassociation kinetics of pooled genomic DNA from a bacterial community. The length of time for reassociation is proportional to the number and relative abundance of distinct sequence fragments (*7*). In 1990, landmark reassociation studies with bacterial community DNA provided the basis for the now widely accepted paradigm of "10,000 bacterial species per gram soil" (*6*). However, genetic diversity has been grossly underestimated as a result of the use of an analytical approach that implicitly assumes all bacterial species in a sample are equally abundant.

Using previously published data for community DNA from pristine and metal-contaminated soils (*8*), we demonstrated an approach that enables quantitative comparison of different species-abundance models. The original reassociation study was performed to assess the effects of heavy metal pollution in soil caused by the repeated application of sewage sludge (*8*). The authors purified bacterial cells from soil samples, extracted DNA from the bacterial cells, extensively purified the DNA by repeated hydroxyapatite chromatography, and then monitored DNA reassociation in sealed cuvettes by optical absorbance (*8*).

When all sequences are equally abundant, the reassociation of DNA, monitored spectroscopically, follows pseudo-second-order reaction kinetics (*9*). For samples containing DNA fractions that differ in relative abundance, a modified version of the basic equation for reassociation kinetics was developed that allows n fractions (abundance classes) with different reassociation rates but does not enable direct comparison of different abundance models (*10*), i.e.

$$\frac{[C]}{[C_0]} = \frac{\sum_{i=0}^{n-1} \frac{f_i}{(1 + k_i[C_0]t)^\gamma}}{\sum_{i=0}^{n-1} f_i} \quad (1)$$

where $[C]$ is the concentration of single-stranded DNA, $[C_0]$ is the initial concentration of single-stranded DNA, t is time, γ (the "retardation factor") is a heuristic DNA-sequence-independent constant (*9*), k_i and f_i are the reassociation rate and relative abundance of the ith DNA fraction, and n is the number of fractions.

We recast this equation in terms of the total number of species (S_t) in a community and the

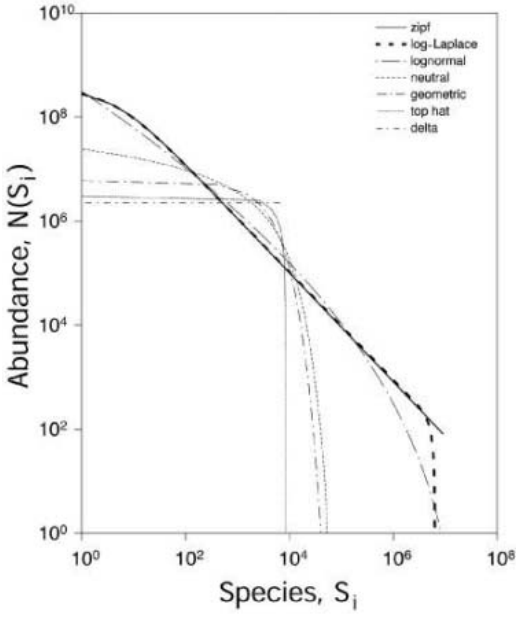

Fig. 3. Rank abundance plots of each model for the noncontaminated soil. Species are ranked in order from most abundant to least abundant.

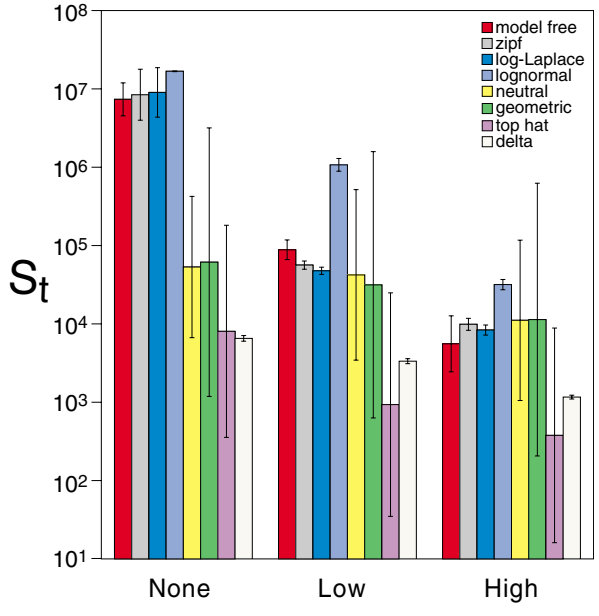

Fig. 4. The total number of species (S_t) computed for the noncontaminated, low-metal, and high-metal soil samples.

relative abundance of each species to allow direct comparison of different abundance models. Using a number of substitutions and approximations (*11*), we obtained

$$\frac{[C]}{[C_0]} = \int_0^\infty \frac{NP(N)dN}{\langle N\rangle(1 + \beta N[C_0]t)^\gamma} \qquad (2)$$

where N is the number of individuals, $P(N)dN$ is the normalized species-abundance distribution, $\langle N\rangle$ is the average number of individuals per species, and β is the ratio of the reassociation rate of a reference genome (e.g., *Escherichia coli*) and the total number of individuals (N_t) in a sample.

The evaluation of Eq. 2 requires values for β and γ (*11*) and an a priori form for the species-abundance distribution. In the absence of a strong justification for a particular distribution, we adopted a variety drawn from macroecology (*11*). To provide a relatively unbiased, heuristic estimate of $P(N)$, we also compared a piece-wise linear approximation

$$P(N)dN = \left[N_0(\Delta - 1)\sum_{i=0}^{n-1} p_i\Delta^i\right]^{-1} \times \sum_{i=0}^{n-1} p_i\left[\theta\left(N - N_0\Delta^i\right) - \theta\left(N - N_0\Delta^{i+1}\right)\right]dN \qquad (3)$$

This "model-free" approximation has the form of a histogram with geometric bar widths. There are n bars, with heights p_i and widths $\Delta^i N_0$, where N_0 is the location of the left edge of the first bar and θ is the Heaviside step function. This yields $n+2$ free parameters to be determined by fitting Eq. 3 to experimental Cot curves. The model-free approximation provides a more flexible shape that does not require symmetry or continuity like the standard abundance models and consequently provides a useful baseline for assessing the fit of standard models.

Using this framework, we reanalyzed the three published (*8*) reassociation data sets for bacterial communities. Two observations were noteworthy. First, we were able to describe the general shape of the abundance distribution. Second, we were able to estimate improved boundaries for the total amount of genetic diversity.

Empirical data and simulations both demonstrate that DNA reassociation kinetics can accurately identify different abundance patterns, although the resolving power depends on the completeness of the reassociation curve. For example, a delta function (a distribution in which all components, e.g., genes, are equally abundant) provided the best fit (as expected) for experimental, single-species *E. coli* DNA reassociation curves. For contrast, we simulated DNA reassociation for a theoretical bacterial community with 5000 species following a lognormal abundance distribution (*11*). After adding Gaussian noise to the reassociation curve equal to the noise seen in the soil DNA data sets, we fit the curve with a variety of abundance models and compared the fits using χ_ν^2 (i.e., reduced χ^2, which accounts for differences in the number of free parameters between models). Even with a reassociation curve only 50% complete, χ_ν^2 values clearly identified the underlying distribution as lognormal while other models were easily excluded (fig. S1).

For the soil bacterial communities (*8*), the value of χ_ν^2 obtained from fitting each model ruled out the delta, top hat, geometric, and neutral models for the species-abundance distribution (Fig. 1). The lognormal distribution was also discounted because it consistently produced larger χ_ν^2 values than the zipf distribution (*11*). The remaining models were qualitatively similar. For all three soil DNA reassociation curves, the model-free curve provided the best fit (Fig. 1), followed closely by the zipf and log-Laplace models (which were statistically indistinguishable). The fluctuation in the model-free DNA reassociation curve for the noncontaminated soil (Fig. 1) reflected a deviation in the shape of the species-abundance distribution (Fig. 2), not a significant increase in species diversity compared with the zipf model.

The zipf and log-Laplace distributions shared the same power-law form describing the most abundant (large N) bacterial species. The power-law envelope defined by the zipf distribution had the form $P(N) \sim N^z$, where z was approximately –2 ($z = -1.96 \pm 0.02$, -2.11 ± 0.01, and -2.08 ± 0.03 for the noncontaminated, low-metal, and high-metal data sets, respectively). Power laws have de-

scribed the abundance distribution of artificial life forms ($z = -2$, most commonly) (*12*), marine phages ($z = -1.64$ and -1.73) (*13*, *14*), and plant communities (*15*) and may arise from a variety of mechanisms (*12*, *16*, *17*). Alternatively, a log-Laplace distribution, which would appear as a power law when measured by DNA reassociation, may arise from an ensemble of lognormals (*18*, *19*) that individually describe the abundance distribution of different functional groups (e.g., denitrifiers, iron reducers, and sulfate reducers).

The zipf and log-Laplace differed mathematically in describing the rare species (Fig. 3). This difference in the two functions was not apparent in the values of χ^2_ν as a result of the incompleteness of the curves and the magnitude of the measurement error, which masks small changes in the shape. The ambiguous shape of the distribution for rare species demonstrates that a portion of the community is veiled. Although a reasonable estimate can be obtained of the minimum number of species in the community (including the veiled fraction), additional work is required to obtain a fully accurate description of the entire species-abundance distribution.

Although the shape of the abundance distribution is of fundamental importance, the total diversity is often of greatest interest in environmental assessment and regulatory policy. For each soil, the model-free, zipf, and log-Laplace estimates of S_t agreed within a factor of two (Fig. 4). Given the qualitative and quantitative similarity of these distributions, we averaged the three to obtain an estimate for each soil. Thus, the noncontaminated, low-metal, and high-metal soils respectively contained about 8.3×10^6, 6.4×10^4, and 7.9×10^3 species among approximately 10^{10} cells [or 10 g of soil; this represents the quantity of DNA used in the reassociation experiments (*11*)]. Our estimates of S_t were larger by a factor of 4 to 500 than the original estimates of 1.6×10^4, 6.4×10^3, and 2.0×10^3 species.

On the basis of our estimates, metal pollution reduced diversity more than 99.9%. Interestingly, total bacterial biomass remained unchanged at about 2×10^9 cells per gram of soil despite metal exposure (*8*). Our abundance models were consistent with this observation and indicated that the major effect of metal exposure was the elimination of rare taxa (Fig. 2). In the pristine soil, taxa with abundance values $<10^5$ cells per gram accounted for 99.9% of the diversity, and genetic diversity from this fraction of the community appears to have been purged by high metal pollution. The functional importance of these rare taxa for soil nutrient cycling and ecosystem resilience is unknown.

To assess the overall error for S_t, we calculated the net impact of all error sources, including measurement error, Cot curve completeness, calibration rate, and hybridization of mismatched DNA (*11*). The relatively minor effects of the first two factors were included in the error estimates for S_t shown in Fig. 4 and could be reduced further (fig. S3). Given that all error sources were random and uncorrelated, the total error for S_t, calculated by standard propagation of errors (*20*), was a factor of 8.2. As this error range affects S_t but is not expected to influence the relative differences between the soils, we are confident of the relative impact of metal pollution.

Comparing the ability of numerous species-abundance distributions to reproduce experimental DNA reassociation data showed that the soil bacterial communities were naturally best represented by the model-free approximation, followed closely by the zipf (i.e., power law) distribution. Hence, the original study substantially underestimated the species diversity of pristine soil bacterial communities. Moreover, heavy metal pollution reduced bacterial diversity not by a factor of 8, as previously suggested, but by a factor of about 1000, with rare species impacted the most. Although the minimum number of species in the soils can be estimated, the exact shape of the abundance distribution for rare species remains ambiguous and is an area for additional work. Overall, the improved analytical approach demonstrates that rigorous DNA reassociation studies can address otherwise intractable problems in microbial ecology, such as monitoring environmental perturbations and mapping diversity geographically.

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Supporting Online Material

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Circadian Clock Control by SUMOylation of BMAL1

Luca Cardone,[1] Jun Hirayama,[1] Francesca Giordano,[1] Teruya Tamaru,[2] Jorma J. Palvimo,[3]* Paolo Sassone-Corsi[1]†

The molecular machinery that governs circadian rhythmicity is based on clock proteins organized in regulatory feedback loops. Although posttranslational modification of clock proteins is likely to finely control their circadian functions, only limited information is available to date. Here, we show that BMAL1, an essential transcription factor component of the clock mechanism, is SUMOylated on a highly conserved lysine residue (Lys^{259}) in vivo. BMAL1 shows a circadian pattern of SUMOylation that parallels its activation in the mouse liver. SUMOylation of BMAL1 requires and is induced by CLOCK, the heterodimerization partner of BMAL1. Ectopic expression of a SUMO-deficient BMAL1 demonstrates that SUMOylation plays an important role in BMAL1 circadian expression and clock rhythmicity. This reveals an additional level of regulation within the core mechanism of the circadian clock.

SUMOylation—the covalent linking of small ubiquitin-related modifier protein (SUMO) to lysine residues—is a reversible posttranslational modification controlled by an enzymatic pathway analogous to the ubiquitin pathway (*1*–*3*). The addition of SUMO on target pro-

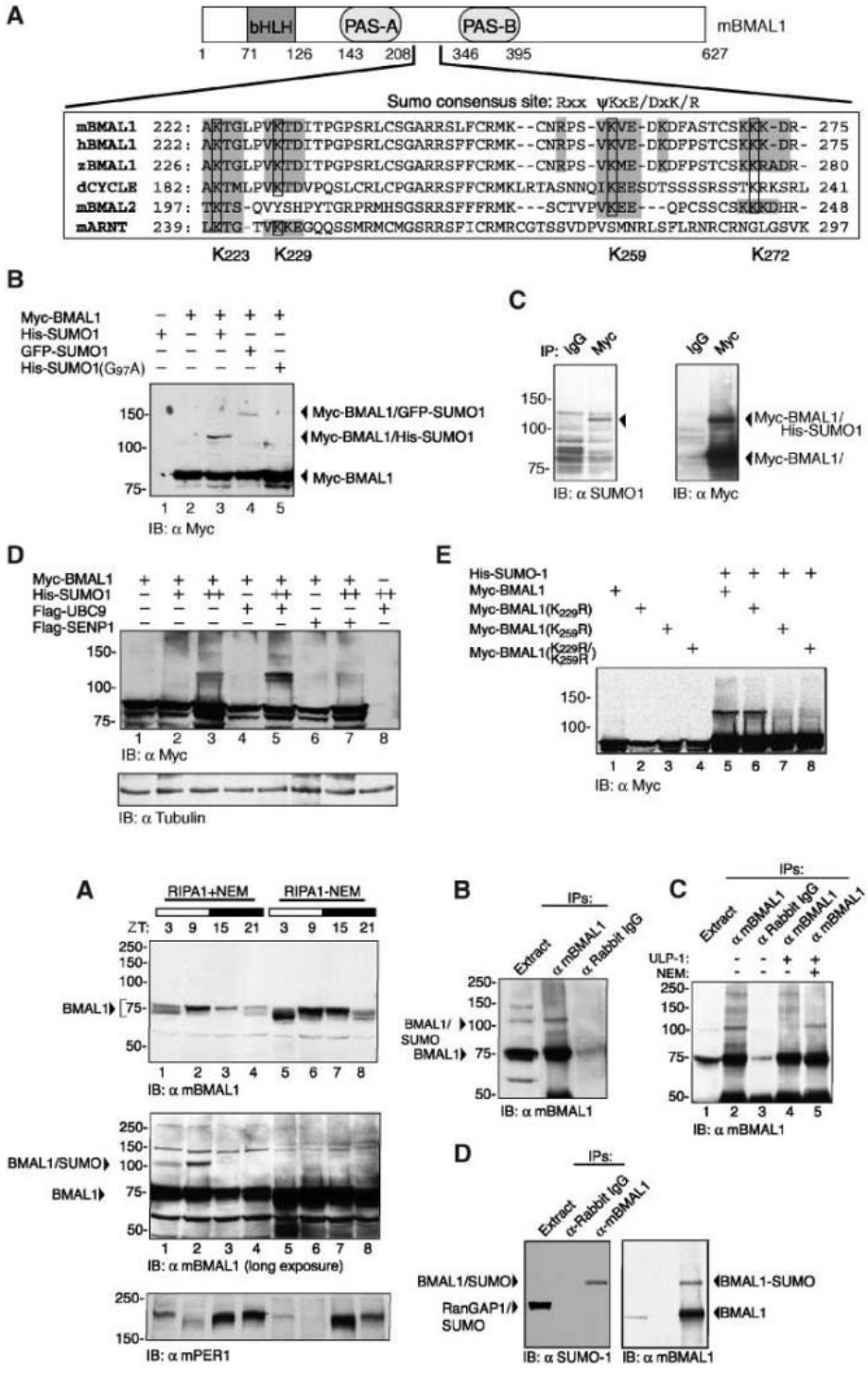

Fig. 1. mBMAL1 is SUMO1 modified in vivo at a highly conserved lysine residue. (**A**) Schematic representation of mBMAL1 and lysine conservation within the linker region between the PAS domains. Conserved lysine residues are boxed. Potential SUMO consensus motifs are highlighted. dCYCLE is the *Drosophila* homolog of BMAL1. mARNT is the mouse ARNT, member of the bHLH/PAS family. (**B**) COS1 cells were transfected with expression vectors for Myc-BMAL1, His-SUMO1, GFP-SUMO1, and His-SUMO1(G97A). Cell extracts were immunoblotted (IB) with an antibody (α) to Myc. (**C**) (Left) Lysates from cells expressing Myc-BMAL1 and His-SUMO1 were immunoprecipitated with anti-Myc or control immunoglobulin G (IgG) and then revealed by Western analysis with an antibody to SUMO1. (Right) The same membrane from the left panel was stripped and probed with anti-Myc. (**D**) COS1 cells were transfected with expression vectors for Myc-BMAL1 (0.25 μg), His-SUMO1 (+, 0.5 μg; ++, 1 μg), Flag-UBC9 (1 μg), and Flag-SENP1 (1 μg) and protein extracts were immunoblotted with antibody to Myc. The lower panel shows an anti–α-tubulin immunoblot from the lower part of the same membrane. (**E**) Immunoblot with an antibody to Myc of protein extracts from COS1 cells expressing BMAL1 wild type or Lys→Arg mutants and His-SUMO1 as indicated. K^{259} is the major SUMOylation site in BMAL1.

Fig. 2. Circadian SUMOylation of BMAL1 in mouse liver. (**A**) (Top) Equal protein amounts from mouse liver extracts at the indicated zeitgeber time (ZT) prepared in radioimmunoprecipitation assay (RIPA1) buffer with or without NEM inhibitor were immunoblotted (IB) with an antibody (α) to mBMAL1 (*32*). The light-dark schedule entraining is indicated by the white and black bars. (Middle) A longer exposure of the above membrane shows the NEM-stabilized form of BMAL1 at about 100 kD (BMAL1-SUMO). (Bottom) Anti-mPER1 immunoblot on the same liver extracts. (**B**) Immunoprecipitates from NEM-supplemented liver extracts (ZT9) using an antibody to BMAL1 or control IgG, revealing native proteins using an antibody to BMAL1 for Western blot analysis. (**C**) The immune complexes obtained as in (B) were incubated with 2 units of recombinant ULP1 protease and analyzed by anti-BMAL1 immunoblot. Inhibition by NEM (5 mM) was obtained by preincubation of ULP1 before the cleavage assay. A lower exposure of this immunoblot shows that equivalent amounts of BMAL1 were immunoprecipitated from each sample (fig. S8). (**D**) Anti-BMAL1 and control immunoprecipitates obtained as in (B) were probed with an antibody to SUMO1 (left). The same membrane was stripped and analyzed by anti-BMAL1 immunoblot (right).

teins has been implicated in transcriptional regulation by a number of mechanisms (*3*–*6*). A hallmark of SUMOylation is that, for most substrates, only a small fraction of intracellular substrate molecules are modified at any given time. SUMO modification is rendered reversible by SUMO-specific proteases, such as the yeast ubiquitin-like protease type 1 (ULP1) or the mammalian sentrin-specific protease type 1 (SENP1) (*7*, *8*).

BMAL1 is a member of the basic helix-loop-helix (bHLH)/Per–aryl hydrocarbon receptor nuclear transporter (ARNT)–Sim (PAS) domain family of transcription factors which, together with its heterodimerization partner CLOCK, drives the circadian clock mechanism (*9*–*11*). Between the two PAS domains, which mediate CLOCK:BMAL1 dimerization (*12*), there is a linker region of undetermined function. Some specific lysine residues in this region are well conserved across species and among different isoforms of the BMAL1 family (Fig. 1A). Because lysines are targeted by multiple posttranslational modifications (*13*), we hypothesized that these residues could constitute regulatory sites. Computer modeling analysis has revealed that the best match for K^{223}, K^{229}, K^{259}, and K^{272} (*14*) in mouse BMAL1 (mBMAL1) corresponds to the SUMOylation consensus motif ψKxE/D (*3*), where ψ is a hydrophobic residue and x may be any amino acid (Fig. 1A).

To determine whether BMAL1 could be SUMO modified in vivo, we transiently expressed epitope (Myc)–tagged BMAL1 in COS1 cells (Fig. 1B). When Myc-BMAL1 was coexpressed with His-tagged SUMO1 or a fusion protein containing green fluorescent protein and SUMO (GFP-SUMO1), we observed Myc-BMAL1 species of higher molecular weight, proportional to the size of additional SUMO modification. No modification of Myc-BMAL1 was observed by coexpressing His-SUMO1[$G^{97}\rightarrow A^{97}$ (G97A)], a single–amino acid mutant that is unable to be transferred onto target substrates (*15*). SUMOylation was further confirmed when immunoprecipitated Myc-BMAL1 reacted with antibodies to SUMO (Fig. 1C). BMAL1 modification was enhanced upon coexpression of ubiquitin-like conjugating enzyme 9 (UBC9), a SUMO conjugating type enzyme (E2) ligase that activates the SUMOylation pathway (*3*). Conversely, coexpression of the SUMO1-specific protease SENP1 (*8*) reduced BMAL1 SUMOylation (Fig. 1D and fig. S1A). Site-directed mutagenesis of the four consensus lysine residues in either Ala or Arg, alone or in combination, demonstrated that K^{259} is the major in vivo SUMOylation site (Fig. 1, A and E, and fig. S1B) (*16*). Importantly, K^{259} is placed within the larger SUMO consensus motif RxxVKVExK (Fig. 1A) (*17*). In vitro SUMOylation assays confirmed that BMAL1 is modified by SUMO1 specifically at the K^{259} residue (fig. S1C). BMAL1 can also be modified by SUMO2 in vivo at the K^{259} residue, comparable to modification by SUMO1 (fig. S2).

To establish the relevance of BMAL1 SUMOylation in circadian physiology, we analyzed the peripheral clock in the liver (*10*, *11*, *18*). Liver tissues from mice entrained at different zeitgeber times (ZT) were collected and protein extracts were prepared in the presence or absence of *N*-ethylmaleimide (NEM), an inhibitor of SUMO proteases that stabilizes SUMO-modified proteins (*19*) (Fig. 2). As previously reported (*20*, *21*), BMAL1 shows rhythmicity in protein abundance and phosphorylation, with hyperphosphorylated forms at ZT9 and at ZT15 and lower protein levels at ZT21 (Fig. 2A, top). NEM stabilized a BMAL1 SUMOylated form (about 100 kD), the abundance of which oscillated in a circadian manner, with a peak at ZT9 (Fig. 2A, middle). No NEM-stabilized forms were detected at ZT15 and ZT21 (fig. S3), whereas no SUMOylated BMAL1 was detected in the absence of NEM (Fig. 2A, lanes 5 to 8). As a control, we examined the expression of the Period 1 (PER1) clock protein from the same extracts (Fig. 2A, bottom). We confirmed the identity of the 100-kD NEM-stabilized form by immunoprecipitation using an antibody to mBMAL1 (Fig. 2B). The effect of NEM was verified by analyzing the levels of SUMO-modified RanGAP1 in the same extracts (fig. S4). The 100-kD SUMO-BMAL1 from liver extracts was readily cleaved by the SUMO-specific protease ULP1 (*7*), in a NEM-sensitive manner (Fig. 2C, lanes 4 and 5). Similar results were obtained when the recombinant ULP1 used here was tested on SUMO-RanGAP1 (fig. S5). In vivo SUMOylation was confirmed when the immunoprecipitated 100-kD BMAL1 form reacted with an antibody to SUMO1 (Fig. 2D). Finally, the rhythmic SUMOylation of BMAL1 is not likely due to oscillations in the *Sumo* genes (fig. S6). These findings indicate that BMAL1 undergoes rhythmic SUMOylation in vivo, with a timing that parallels BMAL1 circadian activation.

BMAL1 heterodimerizes with the transcription factor CLOCK to induce the transcription of target genes (*9*–*11*, *22*). CLOCK also induces BMAL1 phosphorylation (*23*) by a yet unidentified mechanism. Here, we find that BMAL1 phosphorylation and SUMOylation increase in

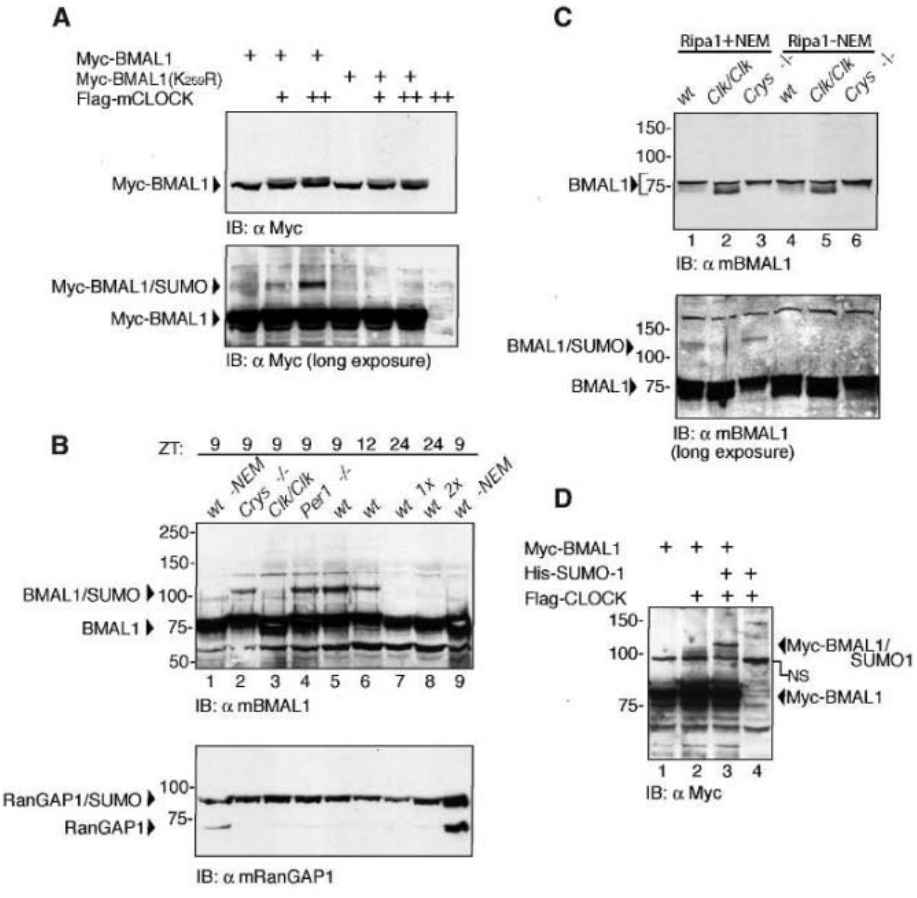

Fig. 3. CLOCK induces BMAL1 SUMOylation in vivo. (**A**) (Top) COS1 cells were transfected with expression vectors for Myc-BMAL1, Myc-BMAL(K259R) mutant, and Flag-mCLOCK, as indicated, and cell extracts were immunoblotted (IB) with an antibody (α) to Myc. (Bottom) Longer exposure of the same membrane. (**B**) (Top) Anti-mBMAL1 immunoblot of equal amount of total liver extracts derived from wild-type (wt), *Cry1*$^{-/-}$/*Cry2*$^{-/-}$ (*Crys*$^{-/-}$), *Clock*/*Clock* (*Clk*/*Clk*), and *Per1*$^{-/-}$ mice collected at indicated zeitgeber times (ZT) in RIPA1 buffer. As controls, protein extracts prepared without strong detergent and NEM (lane 1) or without NEM (lane 9). In lane 8, twice (2×) the amount of protein was loaded. (Bottom) The upper membrane was stripped and immunoblotted with an antibody to RanGAP1. Immunoprecipitations with an antibody to mBMAL1 confirm that SUMO-BMAL1 is not detected in liver extracts from *Clock*/*Clock* mice (fig. S11). (**C**) (Top) Western blot analysis with an antibody to BMAL1 of total protein extract prepared with or without NEM from MEFs derived from wild-type, *Clk*/*Clk*, and *Crys*$^{-/-}$ mice. (Bottom) Longer exposure of top panel reveals the SUMOylated BMAL1 form sensitive to NEM. (**D**) Western analysis of protein extracts from *Clock*/*Clock* MEFs with an antibody to Myc. MEFs were transiently transfected with expression vectors for Myc-BMAL1, His-SUMO1, and Flag-CLOCK. NS, nonspecific. The SUMOylated form of BMAL1 is indicated. Transient expression of CLOCK in *Clock*/*Clock* MEFs rescues BMAL1 SUMOylation.

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parallel at ZT9, concomitantly to the induction of *Period* gene expression (fig. S7). This may indicate that the CLOCK:BMAL1 interaction could trigger phosphorylation and SUMOylation of BMAL1, events possibly coupled to circadian gene activation.

To investigate the role of CLOCK in the SUMOylation of BMAL1, Myc-BMAL1 or the Myc-BMAL1(K259R) mutant was coexpressed with increasing amounts of CLOCK. CLOCK induced BMAL1 SUMOylation in a dose-dependent manner. The Myc-BMAL1(K259R) mutant was refractory to the effect elicited by CLOCK (Fig. 3A and fig. S9).

The presence of SUMO-BMAL1 in the liver from wild-type mice was compared with that in mice carrying mutations in Clock proteins (Fig. 3B and fig. S10). A number of factors contribute to the autoregulatory loops that constitute the clock mechanism (*22*, *24*). These include the products of the *period* genes, which are positively regulated by CLOCK:BMAL1 (*25*), and the *Cryptochrome* genes *Cry1* and *Cry2*, the products of which act as potent repressors (*26*). Whereas neither the disruption of the *Per1* gene (*27*) nor the combined mutation of the *Cry1* and *Cry2* genes (*28*)

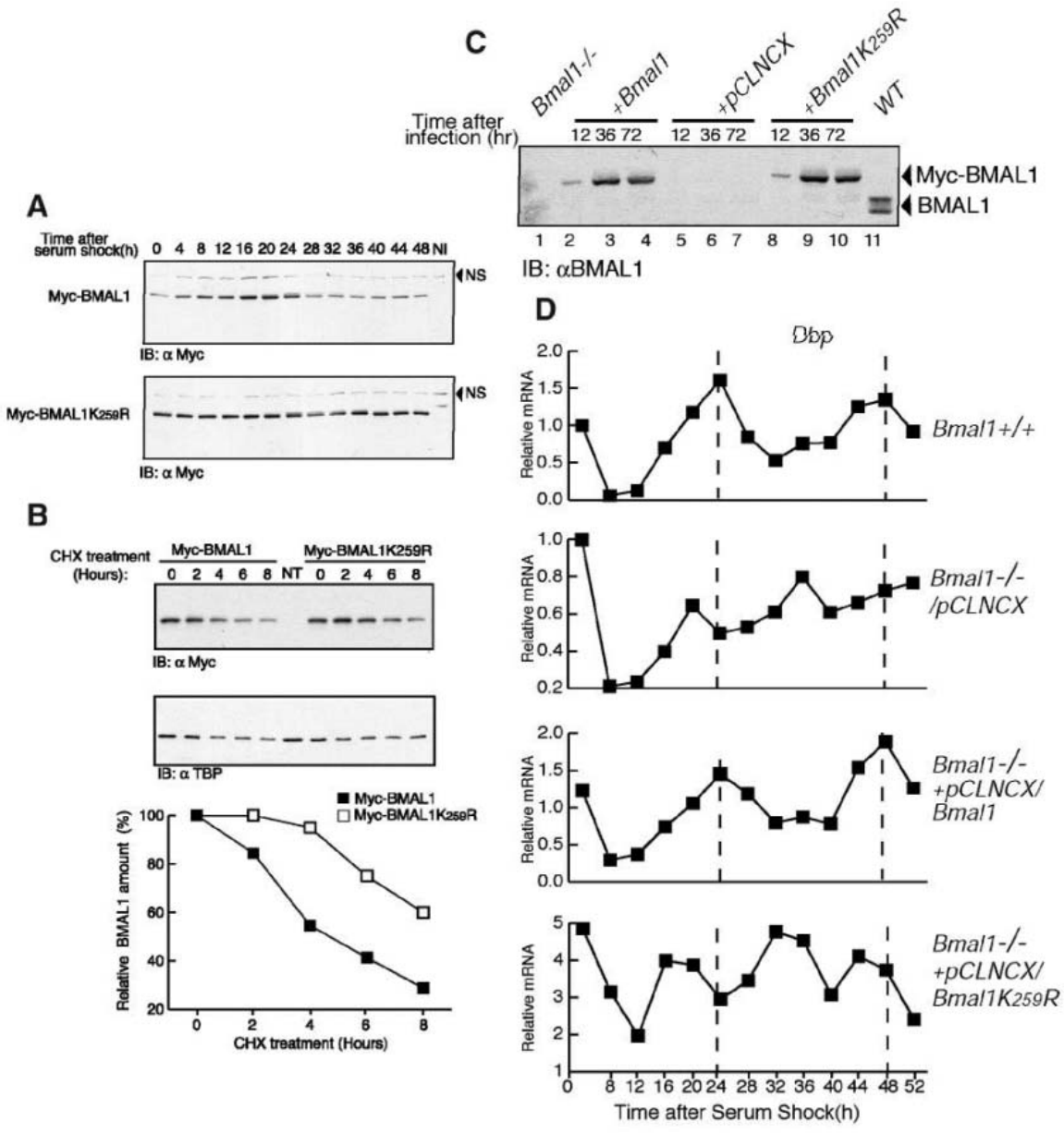

Fig. 4. SUMOylation of BMAL1 controls the molecular clock. (**A**) Antibody (α) to Myc immunoblot (IB) of whole-cell lysates from NIH3T3 cells infected with retroviral vectors expressing either wild-type (top) or a K259R mutated Myc-mBMAL1 (bottom) under the control of the *mBmal1* promoter (*32*). Equal infection efficiencies were obtained for both viruses, as revealed by quantitative analysis of neomycin-resistant (NEO^r) gene expression (*16*). At time T = 0, serum-starved cells were shifted to medium containing 50% horse serum and collected at the indicated time points after serum shock. NI, not infected cells; NS, nonspecific band. Results were quantified and normalized with the anti–TATA-binding protein (TBP) immunoblot performed on the same membrane. The results are representative of three independent experiments, which gave equivalent results. (**B**) Myc-BMAL1(K259R) shows increased protein stability. NIH3T3 cells were transfected with equal amounts of Flag-CLOCK along with Myc-BMAL1 or Myc-BMAL1(K259R). At 36 hours after transfection, cells were treated with cycloheximide (CHX) at 50 μg/ml. At indicated times, cells were lysed and protein extracts were immunoblotted with antibody to Myc (top) and antibody to TBP (middle) as loading control. NT, not-transfected cells. Analogous results were obtained in COS1 cells (fig. S12). (Bottom) The immunoblots were quantified by densitometric analysis. The graph shows the percentage of protein amount relative to T = 0 (100%). The results are representative of three independent experiments, which gave analogous results. (**C**) Ectopic expression of BMAL1 (lanes 2 and 3) and BMAL1(K259R) mutant (lanes 8 to 10) in $Bmal1^{-/-}$ MEFs. Expression levels of BMAL1 protein were evaluated by anti-BMAL1 immunoblot at the indicated times after infection with retroviral vectors as in (A). Protein extracts from MEF cells infected with an empty vector (lanes 5 to 7), wild-type MEFs (lane 11), and $Bmal1^{-/-}$ MEFs (lane 1) were also analyzed as control. (**D**) Lack of SUMOylation alters the serum shock–dependent oscillation of *Dbp*, an E-box–controlled gene. Wild-type and $Bmal1^{-/-}$ MEFs were subjected to a serum shock 2 days after infection with the indicated retroviral vectors. At the indicated time, cells were harvested, and *Dbp* expression levels were estimated by quantitative real-time polymerase chain reaction. Values were normalized to the expression of *Sumo-3*, a nonoscillating gene (fig. S6), and plotted as relative fold of *Dbp* expression at ZT0 (set as 1) in wild-type MEFs. Mean values of four independent experiments are shown, with relative distance from average never above 5%.

(*Cry1*$^{-/-}$/*Cry2*$^{-/-}$) generated reduction in SUMO-BMAL1 levels, there was a notable effect on BMAL1 SUMOylation in *Clock*/*Clock* mice (*29*). This effect was specific to BMAL1; there were no differences in the levels of SUMO- RanGAP1 in the same extracts (Fig. 3B, bottom). Moreover, no SUMO-BMAL1 was detected in mouse embryo fibroblasts (MEFs) derived from *Clock*/*Clock* mice (Fig. 3C), showing that the effect exerted by CLOCK is not restricted to the liver. SUMOylation of BMAL1 was rescued by transient expression of the wild-type *Clock* gene into MEFs from *Clock*/*Clock* mice (Fig. 3D, lane 3), confirming that a functional CLOCK protein is essential for SUMO modification of BMAL1. Interestingly, because the mutant CLOCK protein present in *Clock*/*Clock* mice is still able to heterodimerize with BMAL1 and bind DNA (*29*), SUMOylation may be an event downstream from transcriptional activation.

To determine the function of BMAL1 SUMOylation, we generated a retroviral expression system for Myc-BMAL1 or Myc-BMAL1(K259R) under the control of *Bmal1* promoter. After infection, NIH3T3 cells were serum-shocked to analyze the rhythmic oscillation of BMAL1 expression. This approach recapitulates the circadian regulation of the clock in cell culture (*30*). Whereas wild-type BMAL1 expression peaked at 20 hours after serum shock, with a second peak of lower amplitude at 44 hours, the Myc-BMAL1(K259R) mutant displayed no circadian oscillation (Fig. 4A). These data support the notion that SUMO modification is required for BMAL1 rhythmicity. Whether under the regulation of the *Bmal1* promoter or of a heterologous promoter, the Myc-BMAL1(K259R) protein was about twice as abundant as Myc-BMAL1 (fig. S1B), indicating that lack of SUMOylation may affect protein turnover. Protein half-life analysis by cycloheximide treatment experiments revealed that Myc-BMAL1(K259R) shows an average 50% increased stability compared with that of wild-type BMAL1 (Fig. 4B). Unlike BMAL1, the decay curve of BMAL1(K259R) is not exponential, suggesting a complex degradation mechanism for a protein that is not SUMOylated.

To establish whether BMAL1 SUMOylation is involved in clock function, we undertook rescue experiments using MEFs generated from *Bmal1*$^{-/-}$ mice (*31*). These cells showed no BMAL1 expression (Fig. 4C, lane 1), compared with MEFs generated from wild-type mice (lane 11). *Bmal1*$^{-/-}$ MEFs were readily infected with the retrovirus vectors (Fig. 4A), expressing either BMAL1 (Fig. 4C, lanes 2 to 4) or the BMAL1(K259R) mutant (Fig. 4C, lanes 8 to 10). To study circadian rhythmicity, we scored for the serum shock–induced expression of endogenous *dbp*, an E-box controlled gene (*30*). Although wild-type MEFs showed consistent *dbp* circadian oscillation, this was altered in *Bmal1*$^{-/-}$ MEFs (Fig. 4D). Infection of *Bmal1*$^{-/-}$ MEFs with a virus expressing BMAL1 rescued circadian expression of *dbp*, whereas expression of the BMAL1(K259R) mutant protein generated a shorter period. This was likely due to an increased stability of a BMAL1 protein that cannot be SUMOylated, which in turn could influence the rhythmic expression of other clock proteins.

Our findings provide insight into the mechanisms that control the circadian levels of BMAL1 expression. Several mechanims could be proposed for such differences, including a direct interplay between SUMO modification and the yet unidentified BMAL1 degradation pathway or a SUMO-dependent interaction with partners that control BMAL1 stability (including CLOCK).

SUMOylation of BMAL1 constitutes another level of control within the core circadian clock. Other clock proteins may undergo SUMO-modification in domains distinct from the PAS linker region. Unique elements of the SUMO pathway may be selective for the circadian clock machinery. The recent discovery and characterization of E3 SUMO ligases (*3*) may provide key tools to address these questions.

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32. Materials and methods are available as supporting material on *Science* Online.

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E-mail: r.schatz@neu.edu

Center for Drug Discovery: Medicinal Chemistry & Drug Discovery

The Center for Drug Discovery (CDD) invites applications from outstanding students for predoctoral studies involving the design and synthesis of novel biologically active compounds and studies on their mechanisms of action using biochemical, biophysical and pharmacological approaches. Research includes target-based drug design; receptor purification, characterization and in vivo imaging; and drug-receptor interactions using NMR and computational methods. Concentrations are available in: synthetic, biochemical/pharmacological, and biophysical medicinal chemistry. The CDD's excellence in teaching has been recognized by the award of a training grant from the National Institute on Drug Abuse for predoctoral and postdoctoral training in development of medications. These will be targeted to treat drug abuse and other indications such as neuropathic pain, obesity and neurodegenerative disorders (e.g., multiple sclerosis and Alzheimer's Disease). Graduate students will undertake course work and research leading to a Ph.D. degree in either chemistry and chemical biology or pharmaceutical sciences.

Admissions Information: Ph.D. applications for Fall 2006 must be received by January 15, 2006. The GRE examination, and the TOEFL exam (minimum score of 250, 600 for paper exam) for applicants whose first language is not English, are required.

Contact: Dr. Alexandros Makriyannis or Dr John Gatley, Center for Drug Discovery, 116 Mugar Life Sciences Bldg., Northeastern University, Boston, MA 02115.
E-mail: a.makriyannis@neu.edu.

Careers and Graduate Programs for B.S. & M.S. Scientists: **MORE WITH LESS**

In the world of science, a doctoral degree opens doors. Some scientists might even think that certain doors can be opened only with a Ph.D. Nonetheless, someone with a Bachelor's or Master's degree—who follows the right career path—can often go just as far as a Doctor. The key, of course, is knowing which paths offer the best opportunities. Likewise, a life scientist must know how to get on those rewarding trails.

This article examines career opportunities in academia, government, and industry for someone with a Bachelor's or a Master's degree. In addition, the experts interviewed here stretch from the United States to Europe. So the career opportunities examined in this article cover several disciplines and countries.

Basic Training

With a Bachelor's degree in life science, some career opportunities already appear. The possibilities, though, can be a bit limited, according to Kevin Carman, dean of basic sciences at Louisiana State University in Baton Rouge, but it really depends on the particular field. He says, "In general with biology, chemistry, or physics, one option is teaching." In some states, including Carman's Louisiana, high school teachers now need a degree in the subject that they teach, in addition to a teaching certification.

Carman also points out that someone with a Bachelor's degree can work as a technician in an academic or government laboratory. Nonetheless, he adds, "People are usually looking for someone with a Master's degree, and the top of your career comes fairly early." Still he says, "I've known many techs in labs who love being part of research and make great contributions. They can make a decent living and enjoy what they are doing."

Sometimes, even a Bachelor's degree attracts employers to new graduates. For example, if someone gets a Bachelor's degree in geology from a school with ties to the petroleum industry, recruiters could come calling. Carman says, "Those programs tend to have pipelines to industry." Likewise, petrochemical companies come to Louisiana State University to recruit B.S.-level chemists and chemical engineers.

Many life science undergraduates, though, see the world as composed of narrow opportunities—mostly medical or dental school. Still Carman says, "There are tremendous opportunities in biotech and environmental science." He adds, "Ecotourism is a burgeoning industry. There are many opportunities in biology beyond medicine."

Pick a Profession

If a student goes a couple more years and earns a Master's degree, that can make someone a professional in many areas. For example, a Master's degree in geology is the degree of choice for that field according to Carman. Likewise, a Master's degree in medical physics puts someone in demand. Carman says, "These people come out making six figures. Many of them even get hired after just doing their course work." A Master's degree in other fields, such as computer science and forensic science, also opens doors. Carman says, "We are experimenting with a specialized program where we train state crime lab people for an M.S. in forensic science. In the wake of September 11, there are lots of opportunities."

In reality, a wide variety of degrees spawn careers. Harvey S. Borovetz, professor and chair of the department of bioengineering at the University of Pittsburgh, says, "My experience is that a technical degree opens many doors for graduates." For example, Borovetz has seen people with an undergraduate degree in bioengineering go on to graduate school, medical or law school, or an M.B.A. He says, "A technical background is all very positive."

Borovetz also sees bioengineering students—those with a B.S. or an M.S.—go to work in government agencies, such as the U.S. Food and Drug Administration. Borovetz says, "With bioengineering, government agencies find students at the interface of industry and medicine who have unique backgrounds for regulatory issues and who can interface with patient care and patient health."

National Opportunities

To find out about opportunities at national laboratories in the United States, the author interviewed B.J. Jones, director of human resources at Sandia National Laboratories. This lab sponsors a wide range of scientific endeavors, including chemistry, geology, hydrology, materials science, optics, and physics. This lab also focuses on real-world problems, such as using biotechnology to minimize chemical and biological terrorism. Jones says, "Sandia developed one of the decontamination formulations used to clean out buildings during the anthrax scare."

This high-powered work includes some room for scientists and engineers with either a B.S. or M.S. Of Sandia's 1,627 CONTINUED »

Life Sciences at Missouri

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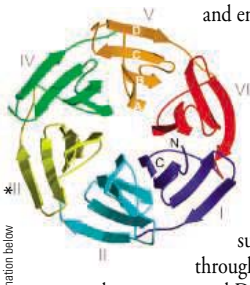

*See information below

Research facilities at MU are outstanding and include the new 200,000 square foot Life Sciences Center, which serves as a hub for state of the art science research and education for the campus community. Centralized support services are available for high throughput genomics and proteomics analyses, automated DNA sequencing and DNA microarrays, multifoton confocal microscopy, scanning and transmission electron microscopy, small animal imaging with MRI and CT, monoclonal antibody production and cell sorting, transgenic and knockout mice and rats, transgenic plants, X-ray crystallography and NMR spectroscopy. The campus is also home to the nation's largest university research reactor.

Commitment to Student Success

Recent PhD graduate Latisha Love-Gregory says it best: "The collaborative spirit of the science community at MU and the interdisciplinary approach to scientific investigation provided me an excellent training environment with great research opportunities. My faculty mentors treated me with respect and their commitment to my success was obvious. The faculty also maintained an open-door policy allowing for impromptu discussions. A "survival course" was presented for new graduate students that emphasized putting together and presenting effective scientific presentations and manuscripts. I felt ready and well-prepared for the new challenges I've encountered in my postdoctoral endeavors because of my experiences at MU."

A Great Place to Live . . .

MU is located in the city of Columbia — a vibrant, well-educated community of diverse cultures. Columbia is conveniently located in the center of the I-70 corridor through Missouri — just 2 hours from Kansas City or St. Louis — and is consistently ranked as one of the nation's most livable cities. Missouri is a beautiful state, with recreational opportunities including the sophistication of big cities, the charm of small towns, and outdoor favorites — hiking and biking trails, lush forests and the clear waters of the Ozark National Scenic Riverways.

*Ribbon drawing of the human Keap1 protein, a redox-regulated substrate adaptor for protein ubiquitination. Courtesy of Dr. Lesa Beamer, MU Dept. of Biochemistry. Published in *Journal of Biological Chemistry*, December 24, 2004, Vol 279:54750-54758.

"The collaborative spirit of the science community at MU and the interdisciplinary approach to scientific investigation provided me an excellent training environment with great research opportunities . . . I felt ready and well-prepared for the new challenges I've encountered in my postdoctoral endeavors because of my experiences at MU."

Latisha Love-Gregory, PhD
Dr. Love-Gregory graduated from MU with a PhD in Genetics and is now a clinical fellow in chemistry and molecular diagnostics at Washington University in St. Louis, Mo.

Doctoral and Postdoctoral Fellowships and Degree Programs

Doctoral degrees are offered in over 40 life sciences departments and programs at MU. A variety of fellowships are available which include a competitive stipend, tuition waiver and health insurance. Graduate students in life science departments may also be eligible to participate in interdisciplinary NIH training grants. Postdoctoral fellowships are available in many departments and programs as well.

A sampling of life sciences programs can be found below. A complete listing of academic programs and campus information can be found at **http://www.missouri.edu**.

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Biological Sciences
http://www.biology.missouri.edu
Phone toll free: (800) 553-5698

Biomedical and Health Informatics Research Training Fellowships
http://www.hmi.missouri.edu
Phone: (800) 877-4764

Comparative Medicine Program
http://www.radil.missouri.edu/info/cmp
Phone: (573) 882-6623

Conservation Biology
http://www.conserv.missouri.edu
Phone toll free: (800) 553-5698

Genetics Area Program
http://www.biotech.missouri.edu/genetics
Phone toll free: (877) Gene-PhD (436-3743)

Interdisciplinary Neuroscience Program
http://www.neurosci.missouri.edu
Phone toll free: (800) 553-5698

Interdisciplinary Plant Group
http://www.plantgroup.org
Phone: (573) 882-4847

Life Sciences Fellowships
http://lifesciences.missouri.edu
Phone toll free: (877) 436-3743

Medical Pharmacology & Physiology
http://www.missouri.edu/~maldz2/pharm/index.htm • **Phone:** (573) 882-4957

Mentored Biomedical Researchers Training Initiative
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Molecular Microbiology & Immunology
http://www.missouri.edu/~mmiwww
Phone: (573) 882-8152

Pathology & Anatomical Sciences
Web: http://www.muhealth.org/~pathology
Phone: (573) 882-1201

Plant, Insect & Microbial Sciences
http://www.plantsci.missouri.edu
Phone: (573) 882-3001

Radiopharmaceutical Sciences Institute
http://www.muhealth.org/~rsi
Phone: (573) 882-2557

Veterinary Pathobiology
http://www.cvm.missouri.edu/vpbio
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BS/MS CAREERS AND GRAD PROGRAMS

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scientists, 19 percent hold a B.S. as their most advanced degree, and 36 percent have an M.S. Of the lab's 2,272 engineers, 12 percent have a B.S. and 54 percent has an M.S. as their terminal degree. Moreover, a B.S. or less is the highest degree for nearly all of Sandia's technician staff. In addition, those with B.S. or M.S. degrees can pursue advancement in non-technical fields such as technology transfer and management. Jones says, "B.S. and M.S. scientists are very important support folks who set up and conduct experiments, collect and analyze data."

Jones says, "A national lab is an interesting animal. It is a combination that looks like academics, government, and industry all rolled into one." For example, the U.S. Department of Energy owns Sandia, Lockheed Martin operates it, and the scientists perform cutting-edge research like academics.


HARVEY S. BOROVETZ

Industry in the U.S.

The pharmaceutical industry in the United States also offers many opportunities for scientists with a B.S. or an M.S. For example, Karen Lewis, associate director of strategic staffing for Bristol-Myers Squibb's Pharmaceutical Research Institute, says, "We have more opportunities for B.S.- and M.S.-level scientists as compared to people at the doctoral level."

At Bristol-Myers Squibb a B.S.-level scientist works mostly at the bench. For example, that might include conducting experiments designed by more advanced employees. Lewis says, "In general, as people advance and build more skills to design experiments then they can advance and take over more independent work in designing and leading others." The same is true at Beckman Coulter. In describing this company, Deb Baxter, group manager of human resources at Beckman Coulter, says, "We are in the immunoassay business. We develop and manufacture the assays that physicians use to diagnose very specific diseases, such as cardiovascular and infectious diseases, cancer, and others." According to Katie Bedney, senior staffing specialist at Beckman Coulter, "Recently, the largest number of Bachelor's-level biologists and chemists have been hired as production scientists, who prepare and build the components of the reagents." To get one of those jobs, lab experience helps. Doing one of the summer internships at Beckman Coulter can give a job applicant an edge, as well. Similar jobs are also available for M.S.-level scientists, and these employees might advance faster than B.S.-level ones, according to Baxter.

To really see the difference between being in the pharmaceutical industry with and without an advanced degree, a scientist needs to walk that walk. Sam McClintock, executive director of pharmaceutical research and development at Merck Frosst Canada, Ltd., did. He started his career in the pharmaceutical industry, took time off in his late 20s to earn his Ph.D., and then returned to pharma. He says, "If you're looking at a career that might start with some exposure to lab work, but long term you are thinking of options in manufacturing or a sales and marketing environment, there could be a place for you with just a B.S. or M.S." He adds, "In many large pharma companies, the minimum requirements for a career in sales and marketing is a B.S. or M.S. followed up by intensive internal training programs."

McClintock regularly sees people with a B.S. or an M.S. who work in a research lab for a few years and then pursue another option, such as marketing, quality management, production management, or sales. McClintock says, "The interesting thing is that in a research environment you are somewhat restricted with a B.S. or M.S., but in other parts of an industrial organization that does not happen at all. So there is little limitation for a successful and rewarding career."


KEVIN CARMAN

A European Education's Edge

The educational and career requirements can vary from one country to the next. According to Katarina Bjelke, director of research and postgraduate education at the Karolinska Institute in Stockholm, Sweden, "If you are not going to be the top scientist or higher level scientist then perhaps you don't need a Ph.D. An M.S. can be good in order to get deeper knowledge and to be able to participate in research here." She adds that a Bachelor's graduate might also be trained for a specific career, such as specially trained lab technicians or nursing. Likewise, a B.S. graduate in Sweden can work in industry, but Bjelke says that an M.S. helps there.

In addition, Stafford Lightman, director of the Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology at the University of Bristol in the United Kingdom, says that someone who wants to go to medical school but fails to get in after a B.S. might consider getting a Master's degree. "If someone is keen to do medicine and cannot get in after the Bachelor's degree, this route might be useful for them," he says.

Also, degree programs can differ between countries. Lightman says, "Students in the U.K. have a much more focused Bachelor's degree and finish this degree younger and with considerably less breadth to their education than their U.S. counterparts. The highest fliers are usually snapped up into first-class Ph.D. programs, but many others will be unsure whether this should be their chosen career CONTINUED »

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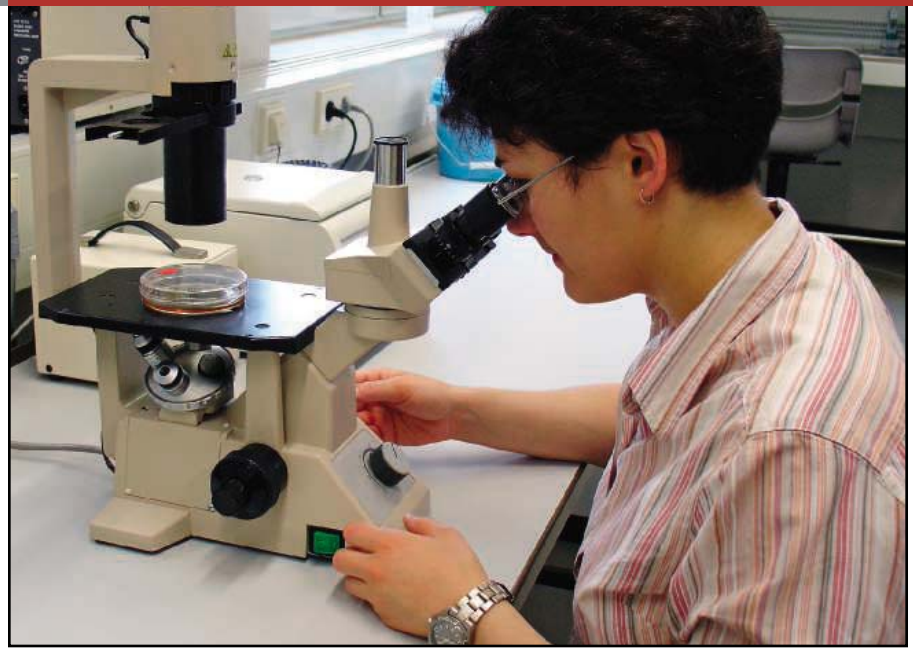

path." He adds, "A Master's degree is a good way to test whether they might be suited to a career in science, as well as a good step onto a good Ph.D. course." He also says that there is a group of M.Sc. graduates who use this degree for business related careers. He says, "The drug industry is a good example. Big companies are interested in people with knowledge of the scientific method."

Lightman does not see many students going into government positions after a Bachelor's or Master's degree earned at the University of Bristol. "That is quite surprising," he says. "The U.K. Ministry of Agriculture Fisheries and Food would appear to be an obvious recipient of these graduates, but we haven't had anyone do that."

Inside Industry in Europe

Much like in the United States, the U.K.'s pharmaceutical industry provides many opportunities for scientists with a B.S. or an M.S. David Lathbury, director of process chemistry at AstraZeneca R&D Charnwood in the United Kingdom, says that a B.S.-level scientist can find exciting prospects inside several areas of the pharmaceutical industry, including patent work, quality assurance, and regulatory affairs. He adds, "Most of these involve extra study. It might not be purely scientific study, but it could include on-the-job training."

Lathbury also sees other industries in the United Kingdom hiring life scientists with a Bachelor's or Master's degree. He says, "The financial services take lots of M.S. and B.S. students, especially those from physical sciences. These businesses need logical, thought-trained graduates." Even in fields that appear specialized, Lathbury sees B.S. graduates getting jobs. For example, he says, "Take analytical chemistry; it is not so heavily dominated by Ph.D.s."

Still, someone with an M.S. degree gets more flexibility. Lathbury says, "Someone with a Master's degree is beginning to straddle the interface between being a generalist and having a research career." He also says, "People with a Master's degree can be better informed on what choices to make in a project, because they have been exposed to some elements of research in their training. They are more independent." Those qualities make someone with a Master's degree more desirable than someone with a Bachelor's degree, even for an entry level position—mostly because the Master's-level applicants probably have a better idea of what research would be like, even in industry. Nonetheless, the starting pay does not vary much based on these degrees.

Picking a Program

Getting to any career related to life sciences, though, depends on some degree. Moreover, earning a career-enhancing degree depends on getting in the right program. But there could also be lots of "right" programs. For future bioengineers, for example, Borovetz of the University of Pittsburgh says, "If you have the opportunity to attend Harvard or MIT, you should obviously consider it. But, you can also receive a wonderful undergraduate education in bioengineering at many colleges and universities—both public and private institutions—across the U.S. At the undergraduate level, students' educational programs mostly focus on the basics." He adds, "That degree allows a student to apply for a job or pursue post-baccalaureate education."

Borovetz sees finding the right graduate program as a more complicated choice. "Graduate school prepares you for the next 30 to 40 years of your professional life," he says. In addition, graduate school takes a different focus than undergraduate work does. Borovetz says, "A prospective graduate student should focus on a school's labs, the research of the faculty, what career paths graduates from various labs have followed." To make that evaluation, Borovetz recommends visiting a variety of schools. "Outstanding students have earned the right to consider top programs," he says. "Strive to be admitted to the top graduate programs, and to undertake Ph.D. research in the lab of a professor whose research excites you."

In the past, many Master's programs in the life sciences aimed most at preparing students for further education—particularly a Ph.D. That is less so today. At the Keck Graduate Institute of Applied Life Sciences, for instance, a student can earn a CONTINUED »

Careers and Graduate Programs for B.S. & M.S. Scientists: **MORE WITH LESS**


DAVID LATHBURY

Master of Bioscience (M.B.S.), which is described on the institute's Web site as "a professional degree and a direct response to leaders in industry, government, and higher education who asked for a new model of graduate science education." Sheldon Schuster, president at Keck, says, "We view a professional Master's degree as a goal unto itself, not a pathway toward further education." Then he adds, "But we always support more education."

A student earns Keck's M.B.S. through a two-year program. This program starts immediately with team projects, which are fundamental elements of the entire program. In addition, this degree requires considerable course work in various areas of science, including the ability to analyze large databases and the application of engineering to problems in life science. Other courses cover finance, management, and even strategies for leading other people. During the summer between the first and second year, a student completes an industry-sponsored internship. Schuster says, "This gives a student real-world experience in a real company."

During the second year of study, an M.B.S. student at Keck takes more courses. At this stage, the course work focuses on a particular track, such as bioprocessing, management, regulatory affairs, or intellectual property. Roughly half of a second-year student's time goes to a team project that serves as a thesis. Working in groups of three to five, students take on a real industry problem. Each team works with a faculty and corporate liaison. The teams also get the necessary facilities, such as lab and office space, and they are expected to create budgets and timelines toward producing some deliverable. "These have been remarkably successful," says Schuster. In some cases, the company even wanted the project to continue, and some students get hired by the sponsoring company.

Schuster also mentions a variety of jobs that graduates take: project management, venture capital analyst, competitive market analysis, and positions in technology transfer. He also says that this M.B.S. prepares graduates for a wide range of possible careers in government, such as working in a patent office or doing technology transfer for national laboratories. He adds, "NIH has an active tech transfer program." Perhaps most important of all, Schuster says, "We see an industry telling us that they want people educated in this matter, and industry is hiring them too."

Whichever program a future life scientist selects, Lathbury of AstraZeneca encourages the development of quantitative skills. He says, "Many of the jobs these days tend to be on the more mathematical side of things." He adds, "Most sciences are getting more quantitative."


B.J. JONES

An Inside Track

As anyone who ever looked for a job knows, a degree is usually not enough—no matter what degree someone holds. Seemingly every advertisement seeks experienced applicants. If a student takes advantage of all of today's opportunities, it is possible to earn a degree and gain experience at the same time. Internships, for example, can provide that experience, and government and industry offer them. Jones of Sandia says, "We have one of the most robust student internship programs that I have ever seen." She says, "It lets students expand their expertise. It also lets them get to know us, and we get to know them." For more information, see Sandia's Student/Special Programs page on the Internet (http://www.sandia.gov/employment/special-prog/index.html).

Lathbury also sees the value of getting some hands-on experience. He says, "It seems that internships are becoming more important. Just six months to a year gives you an edge." He adds, "In the U.K., a student can spend up to a year in industry while getting a degree. These students come out of that process more mature, better trained, and familiar with a job they might apply for in industry. As a result, some companies look for people who have had some time in industry."

At Bristol-Myers Squibb, Lewis also sees experienced applicants excelling. She says, "More applied work experience gives someone more advancement opportunities." This company also helps students gain that experience through a variety of internship opportunities.

Merck also arranges opportunities for advancement in education and creates work experiences for students. For instance, Merck has a program that helps qualified employees earn a Ph.D., even funding the person during the final year or so of the lab work. "We also have graduate students in the lab," says McClintock. "Many members of our staff have appointments with universities." In addition, Merck sponsors summer internships for students and runs a co-op program, in which a student goes to school for a few months, works for a few months, then back to school, and so on. "This has worked for us," McClintock says, "and is a good way to identify potential new employees. This program also provides students with about one year of working experience before they hit the job market, and they also carry out 15 to 20 interviews during this program, which gives them added skills."

To Be or Not to Be a Ph.D.

For some degrees, it can grow difficult to tell one from another. "In the working environment, there tends to be less of a difference between an M.S. and a B.S.," says McClintock. "They tend to get blurred, although the M.S. gives you a bit of a head start getting in the door. Once you are there, though, I don't see much distinction CONTINUED »

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STAFFORD LIGHTMAN

between the two." Then he adds, "The big leap is the Ph.D." The question is: Who should go on for a Ph.D. and who should not?

The range of the answers to that question seems to grow every time that someone else weighs in on the topic. McClintock, for example, says, "For B.S. scientists who are looking for careers that are not lab focused, they are probably better off looking at an M.S. or an M.B.A. rather than a Ph.D."

From his perspective at AstraZeneca, Lathbury says, "Predominantly, for those students who are unclear if they want a deeply scientific career, it is safer to stick with a Bachelor's or Master's degree." He adds, "Some students stayed in for a Ph.D. because they felt like it was a good idea at the time. Invariably that is a mistake." The four to five years of work—often even more—that a Ph.D. requires can rarely be accomplished without passion. Lathbury adds, "Really, only do it if you are truly interested in the subject. You need that drive."

Borovetz at the University of Pittsburgh agrees. He says, "The goal of earning a Ph.D. is to always be at the cutting edge of technology and science. You can't force people to be this way. They have to want it themselves."

Many students, however, go for the Ph.D. in hopes of landing a better job down the road. Lathbury would dissuade a student from that thinking. He says, "You might not get a better job, especially if you don't do well at your Ph.D. You must come out able to compete with your new peers, who will all have Ph.D.s then."

Worse still, Lathbury sees a Ph.D. as a disadvantage in some cases. "Once you have it," he says, "you can't take it away. As a Ph.D. it can be hard to compete with B.S. or M.S. students."

In some places, though, only a Ph.D. will do. For example, those who want to run groundbreaking research generally need the Ph.D. Jones at Sandia says, "The Ph.D. brings the opportunity for original contributions." In addition, the Ph.D. carries considerable clout. Jones says, "The Ph.D. is a form of instant credibility. I may not know you but there is an instant respect." She adds, "In physics, there is a sense that you have not completed your studies without the Ph.D." In addition, Borovetz says that a Ph.D. opens doors in the entrepreneurial world. "If you are developing technologies or therapies and want to spin them out and need to secure funding," says Borovetz, "then signing 'Doctor so and so' likely carries more weight."

A young scientist also faces other limitations without a Doctorate. Carman of Louisiana State University says, "The limitations really depend on your career choice. If you want to go into academics, you need a Ph.D." He adds, "Maybe you could teach at a community college without a doctoral degree, but you would hit the ceiling quickly." The same ceiling


SHELDON SCHUSTER

might not exist in industry or government, according to Carman. Still he says, "To be a head of a government research lab or the head of a research program at a pharmaceutical company, the Ph.D. is almost required." Lewis agrees that having a Ph.D. makes it easier to advance in a scientific specialty at Bristol-Myers Squibb. For those employees interested in pursuing management opportunities, though, she says, "Other factors, such as previously demonstrated leadership skills, come into play."

Earning the Ph.D. can even help in some positions that do not focus on research. Bjelke at the Karolinska Institute earned her Ph.D. in neuroscience and knows that it helped her earn an administrative position. She says, "Even in research administration—either university or governmental ministry—it is becoming increasingly difficult to get a top position in Sweden without a Ph.D." She adds, "The insight gained in a Ph.D. can be applied to other jobs. And in some jobs it is easier to communicate with your colleagues if you have a Ph.D. as a background."

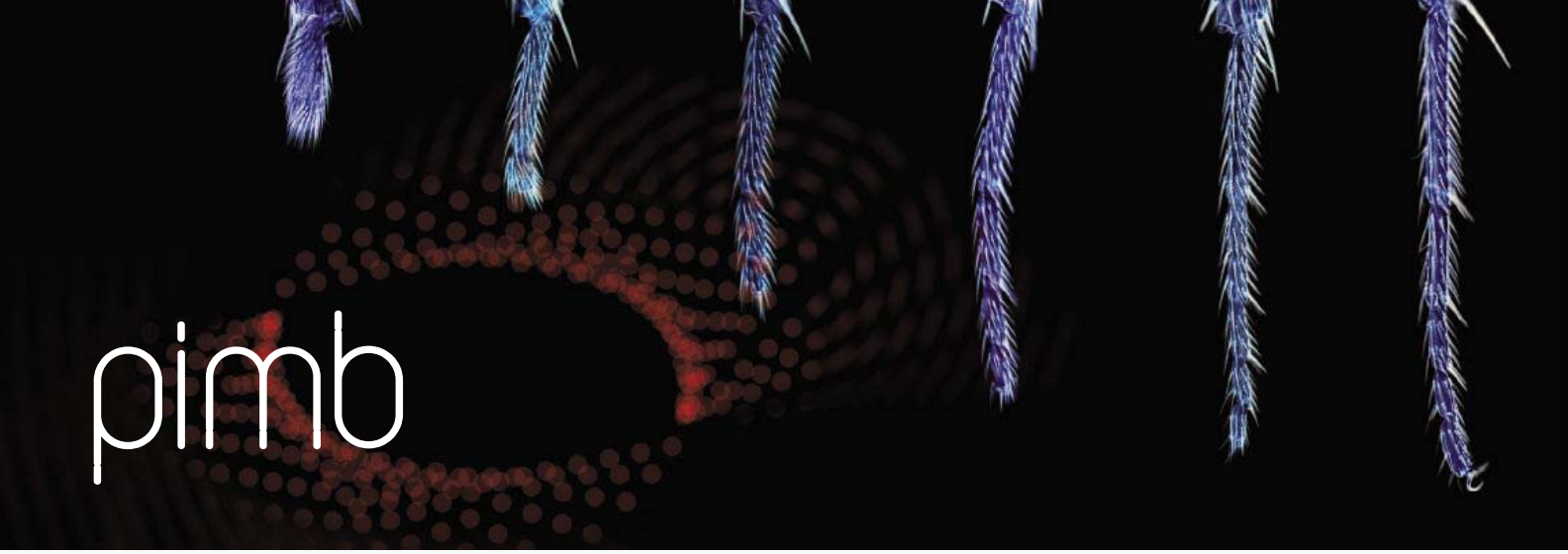
Thinking back on her time doing her doctoral research, Bjelke says, "I had some great years and benefited from that period, even though I didn't become a scientist." Nonetheless, her doctoral degree carried her to a career that she loves. She says, "I still work with, for example, research collaborations, and I love it every day when I go to work."

Staying on Top

In the end, the best path is a personal choice. A B.S. works fine for some scientists, while others cannot rest until they earn a Ph.D. Others still, might prefer to mix and match disciplines and degrees. Carman says, "When we think of what type of degree to pursue in science, one subcategory is the idea of combining science with something else." He explains that an M.S. makes a terrific combination with a law degree. Or a Master's-level scientist could do well in a career in intellectual property. Carman also mentions that a B.S. in science makes a great foundation for a degree in journalism, especially for someone who wants to cover science and technology. These combinations can provide just the right edge to stay on top of a field.

No matter what degree someone earns, the learning is never over. All careers continually demand more knowledge. At Beckman Coulter, for example, employees can request to be paired with a mentor. As Baxter explains, "This helps reveal the lay of the land. It is especially good for new Bachelor's graduates to get the guidance that they need." Beyond internships and mentoring programs, though, every scientist must also be self-motivated, because there is always more to learn.

Mike May (mikemay1@verizon.net) is a publishing consultant for science and technology based in Madison, Indiana, U.S.A.



The Program in Integrative Molecular Biology

Introducing PIMB

The University of Pittsburgh is launching the **Program in Integrative Molecular Biology (PIMB)**, an innovative new course of Ph.D. training for students with a highly focused and developed interest in integrative biology.

Innovative Curriculum

PIMB is designed to quickly immerse students in a challenging research environment and mentor them to become independent scientific practitioners with the creative and technical skills required to address some of the most important questions in the life sciences.

Exceptional Training

Intensive rotations in multiple laboratory environments with diverse technologies and scientific disciplines, specialized coursework, integrated career mentoring, a broad choice of advanced electives, and training in the ethics of scientific exploration are among PIMB's most notable features.

- Class size is limited to 6 to 10 students for optimal interaction with faculty.
- Successful applicants will receive financial support in the form of tuition plus a stipend.
- The program, which will begin in June 2006, is designed for completion in approximately four years. Applications will be received beginning in August 2005.

Outstanding Faculty

An outstanding panel of acclaimed scientists with a broad range of investigative approaches has been assembled from laboratories across the University of Pittsburgh to serve as the faculty for this program.

Susan G. Amara, Ph.D.
Gerard Apodaca, Ph.D.
Karen Arndt, Ph.D.
Ivet Bahar, Ph.D.
Jeffrey L. Brodsky, Ph.D.
Gerard Campbell, Ph.D.
J. Richard Chaillet, M.D., Ph.D.
Yuan Chang, M.D.
Deborah Chapman, Ph.D.
Donald B. DeFranco, Ph.D.
Susan Gilbert, Ph.D.
Paula Grabowski, Ph.D.
Angela M. Gronenborn, Ph.D.
Graham Hatfull, Ph.D.
Roger W. Hendrix, Ph.D.
Jeffrey Hildebrand, Ph.D.
Neil A. Hukriede, Ph.D.
Judith H. Klein-Seetharaman, Ph.D.
Jeffrey Lawrence, Ph.D.
Patrick S. Moore, M.D., M.P.H.
James M. Pipas, Ph.D.
William Saunders, Ph.D.
Thomas E. Smithgall, Ph.D.
Linton M. Traub, Ph.D.
Ora A. Weisz, Ph.D.
Richard D. Wood, Ph.D.

For More Information

Program in Integrative Molecular Biology

Graduate Office
524 Scaife Hall
3550 Terrace Street
Pittsburgh, PA 15261

412-648-8957
PIMBinfo@medschool.pitt.edu
http://www.pimb.pitt.edu

University of Pittsburgh
School of Arts and Sciences
School of Medicine

POSITIONS OPEN

Faculty Position: Broad Institute/ Massachusetts Institute of Technology

The Broad Institute of MIT and Harvard seeks applications for new faculty whose research explores comprehensive approaches to biology and their application to disease.

The Broad Institute is a newly launched collaboration of MIT, Harvard, the Harvard teaching hospitals, and the Whitehead Institute for Biomedical Research. Our mission is to pursue comprehensive approaches to biological systems, with a particular focus on disease biology, enabling scientists to undertake collaborative projects that cannot be readily undertaken in more traditional academic settings. Current areas of activity include genome research in both mammalian and non-mammalian systems, medical and population genetics in human and mouse, cancer genomics, chemical biology, infectious disease, metabolic disease and psychiatric disease.

Faculty appointments at the Broad Institute are made in conjunction with a primary academic department at MIT or Harvard. The appointments include both Core Members, whose laboratories will be located primarily at the institute, and Associate Members, whose laboratories will be located primarily in their primary academic department.

We are currently seeking applicants for a tenure-track joint position as a Core Member of the Broad Institute and Assistant or Associate Professor in the MIT Department of Biology. The successful applicant will lead a world-class research program, have wide-ranging interests in comprehensive approaches to biological systems and an interest in disease biology. Applications are welcomed from scientists working in any of a variety of relevant fields (including molecular biology, genomics, medical genetics, chemistry, computational science, engineering) or at the interface of multiple disciplines, and on either human and model organisms.

Applicants should submit a curriculum vitae, a summary of current and proposed research programs, and should arrange for three letters of recommendation to be sent to: **Biology Search Committee, Attn: Professor Eric Lander, MIT Room 68-132, 77 Massachusetts Avenue, Cambridge, MA 02139.** Consideration of completed applications will begin on **October 15, 2005**.

MIT is an Affirmative Action/Equal Opportunity Employer. Qualified women and minority candidates are especially encouraged to apply.

BS/MS CAREERS AND GRAD PROGRAMS

POSITIONS OPEN

The Children's Hospital at SCOTT & WHITE

College of Medicine
The Texas A&M University System Health Science Center

Endowed Chair
Pediatric Cardiovascular Research
Scott & White Health System
Texas A&M University System Health Science Center
College of Medicine

The **Children's Hospital at Scott & White** and **The Texas A&M University System Health Science Center College of Medicine** are seeking a nationally recognized research scientist as the first holder of the Josephine Ballard Endowed Chair in pediatric cardiovascular research. Applicants should be accomplished investigators (Ph.D., M.D. or M.D./Ph.D.) at the associate or professor level with current federal grants and a proven track record in cardiovascular basic, clinical, and/or translational research. The successful candidate will join an expanding faculty within a large academic healthcare system. The chair holder will play a critical role in directing and expanding research activities in pediatric cardiovascular disease, in close collaboration with investigators in the Cardiovascular Research Institute and other local, national and international experts in cell biology, genomics and proteomics.

The Children's Hospital at Scott & White serves a large clinical base throughout Central Texas. There are outstanding clinical practice and laboratory facilities on campus that perform state of the art molecular and cellular biology techniques, flow cytometry, proteomics and genomics as well as biostatistical support services. Animal laboratory facilities include areas to perform medical and surgical procedures. Laboratory space and an appropriate start-up package for the chair holder will be provided. The Scott & White Healthcare system is one of the largest multi-specialty integrated delivery systems in the nation. Scott & White is the primary clinical and hospital teaching campus for the College of Medicine. Academic appointments at the associate and professor level through the College of Medicine are commensurate with qualifications and experience.

Interested candidates should send a copy of their curriculum vitae, letter addressing their qualifications and a list of 3 individuals who can provide references to: **Don P. Wilson, M.D., Chair, Search Committee for Josephine Ballard Centennial Chair in Pediatric Cardiovascular Research; Chairman, Department of Pediatrics, 2401 South 31st Street, Temple, Texas 76508, 254-724-4363, fax 254-724-1938, email: dwilson@swmail.sw.org**

Scott & White is an equal opportunity employer. For more information regarding Scott & White and The Texas A&M University System Health Science Center College of Medicine, please log onto: www.tamu.edu and www.sw.org.

BS/MS CAREERS AND GRAD PROGRAMS

University at Buffalo
The State University of New York

School of Medicine and Biomedical Sciences

Interdisciplinary Graduate Program in Biomedical Sciences

- **OPPORTUNITY** - discover your interests and talents with faculty doing diverse, cutting edge research, including proteomics, nanomedicine, molecular and systems biology, and bioinformatics.
- **FLEXIBILITY** - an interdisciplinary core curriculum providing fundamental scientific training, with time to pursue individual research interests and select a graduate degree program suiting your career goals.
- **CHOICE** - select among PhD programs in Physiology and Biophysics, Biochemistry, Microbiology and Immunology, Neuroscience, Oral Biology, Pharmacology and Toxicology, Structural Biology, Anatomy and Pathology.
- Tuition waiver and stipend provided

For more information visit:
www.smbs.buffalo.edu/rbe/igpbs/
Or e-mail: **smbs-gradprog@buffalo.edu**

University of Kansas

KU Graduate Program in Molecular Biosciences

Outstanding graduate training is offered in five focus areas:

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- **Genetics**
- **Microbiology**
- **Molecular, Cellular, and Developmental Biology**
- **Neurobiology**

The program provides support for all graduate students, including a competitive 12-month stipend plus tuition.

For a full description of our exciting program, faculty research interests and admission requirements visit:
www.molecularbiosciences.ku.edu

Paid for by KU.

GRADUATE PROGRAMS

Graduate School of GENOME SCIENCE & TECHNOLOGY
The University of Tennessee (UT) & Oak Ridge National Laboratory (ORNL)
http://gst.ornl.gov/

Seeking Outstanding Students

- Full tuition waiver and Health Benefits
- Stipend of $18,000
- Accepting applicants with biological, physical, or computational backgrounds

Interdisciplinary Program

- UT and ORNL facilities/expertise
- Seventy-five faculty
- Academic environment and team approaches

Areas of Study

- Genetics/Genomics
- Structural Biology/Proteomics
- Computational Biology/ Bioinformatics
- Bioanalytical Technologies

To apply visit the website:
http://gst.ornl.gov
Or Write: Dr. Cynthia B. Peterson, Director
UT/ORNL Graduate School of Genome Science & Technology
1060 Commerce Park
Oak Ridge, TN 37830-8026
cbpeters@utk.edu

Positions @ NIH
THE NATIONAL INSTITUTES OF HEALTH

ASSOCIATE DIRECTOR FOR RESEARCH INFORMATICS AND INFORMATION TECHNOLOGY

Immediate Office of the Director
National Heart, Lung and Blood Institute

The National Heart, Lung, and Blood Institute (NHLBI) is seeking a strategic-minded scientist with expertise in research informatics and information technology who will bring significant experience in a research environment to operate in an intellectually challenging Federal biomedical research institution engaged in a national research program to understand, treat, and prevent heart, lung and blood diseases and sleep disorders throughout the world.

This position offers a unique and challenging opportunity for the right individual to work directly with the NHLBI Director to develop a program in research informatics, incorporating information technology. Applicants should possess an advanced science degree and research experience related to bioinformatics or research informatics. Specifically, the successful candidate should have experience in providing bioinformatics support in the areas of biology, molecular biology and genetics, including the terminology of basic, translational, and clinical research. In addition, applicants should have sufficient education and experience that will ensure success in managing a professional and technical staff engaged in providing complex and computationally intense modeling and analytics in the areas of bioinformatics, genomics, proteomics and imaging. It is highly desirable for the successful applicant to also have extensive experience in information technology management, encompassing strategic planning, complex organizational structures, technical project management and process transformation. The successful candidate will serve as the Chief Information Officer for the NHLBI, and will oversee NHLBI science support and administration of NHLBI science in areas such as enterprise operations systems, data warehouse and management reporting and information security and the day to day operations of staff providing IT infrastructure development and support. Strong leadership qualities, negotiation skills and exceptional interpersonal skills are imperative.

Application Process: Salary is commensurate with experience and a full package of Civil Service benefits is available including retirement, health and life insurance, long term care insurance, leave and savings plan (401K equivalent). CV, bibliography and two letters of recommendation must be received by **October 15, 2005**. **Application package should be sent to the National Institutes of Health, attn: Mr. Barry Rubinstein, Building 31, Room 5A-28, 31 Center Drive, MSC 2490, Bethesda, MD 20892-2490. For further information, please contact Mr. Rubinstein by email: Rubinstb@nhlbi.nih.gov or telephone (301) 496-2411**. All information provided by applicants will remain confidential and will only be reviewed by authorized officials of the NHLBI.

The NIH encourages the application and nomination of qualified women, minorities, and individuals with disabilities.

NIH Postdoctoral Research in Proteomics

National Heart, Lung and Blood Institute

A postdoctoral research position is available immediately to apply proteomic techniques to the study of nitric oxide biology in patients with sickle cell disease, including patients with pulmonary hypertension. The postdoctoral fellow will work closely with a team of mass spectroscopists, bioinformaticists and clinical researchers to identify and validate novel biomarkers of pulmonary hypertension. Excellent communication skills are required. A background in protein biochemistry and/or mass spectroscopy is also highly desirable.

Submit applications to:

Gregory J. Kato, M.D.
Director, Sickle Cell Vascular Disease Unit Vascular Therapeutics Section Cardiovascular Branch, NHLBI, NIH
10 Center Dr, MSC 1476 Bldg 10CRC, Rm 5-5140
Bethesda, MD 20892-1476

Phone (301) 451-8497
FAX (301) 451-7091
gkato@mail.nih.gov

Postdoctoral Position

The Metals Section of the Laboratory of Comparative Carcinogenesis at the National Cancer Institute at Frederick invites applications for a Postdoctoral position to work on metal-induced carcinogenesis. The start date is January 2006. The potential candidate must have documented experience in tissue culture, immunohistochemistry, northern and western blotting, reporter assays, RT-PCR, DNA cloning and pinpoint mutagenesis. Preference will be given to candidates who previously worked on metals. Candidate should have less than 5 years postdoctoral experience. Please send curriculum vitae and three letters of recommendations to: **Konstantin Salnikow, Ph.D., National Cancer Institute at Fredrick, Laboratory of Comparative Carcinogenesis, Building 538, Room 205E, P.O. Box B. Frederick, MD 21702, USA. E-mail: salnikow@ncifcrf.gov.**

WWW.NIH.GOV

National Institute Of Diabetes And Digestive And Kidney Diseases

Postdoctoral positions are available in the Liver Diseases Branch (http://intramural.niddk.nih.gov/research/labbranch.asp?Org_ID=610) of NIDDK. Candidates should hold a D.V.M., M.D., Ph.D., M.D./Ph.D., or equivalent degree conferred less than 5 years before beginning the fellowship. Candidates must have a record of outstanding research productivity and familiarity with a wide range of relevant research techniques. The scientific atmosphere of the NIH Intramural Program offers multiple opportunities for education and training. Please send a resume, statement of career goals and names of 3 references to the appropriate investigator.

T. Jake Liang, M.D. (jliang@nih.gov): molecular pathogenesis of virus-cell/host interactions, vaccine development for hepatitis C, animal models for hepatitis B and C, molecular pathways of host antiviral defense, and identifying and characterizing molecular targets for antiviral development.

Caroline C. Philpott, M.D. (carolinep@intra.niddk.nih.gov): This laboratory uses genetic and cell biological approaches to study iron uptake and utilization in eukaryotic cells. Our work in budding yeast has led to the discovery of new genes involved in iron metabolism, novel systems of iron uptake, and unexpected interactions with other metabolic pathways. Newer projects include the use of yeast expression systems to identify novel human genes of iron metabolism.

Barbara Rehermann, M.D. (Rehermann@nih.gov); Basic and clinical immunology research on the pathogenesis of hepatitis B and hepatitis C virus infection and immune-mediated liver disease. Areas of interest include mechanisms of virus-host interaction, correlates of spontaneous and treatment-induced recovery and mechanisms of disease pathogenesis and progression using immunological, molecular and biochemical techniques and experimental animal models.

Marc Ghany M.D. (marcg@intra.niddk.nih.gov): Basic and clinical research on the pathogenesis and therapy of hepatitis B and C . Areas of interest include molecular biology of hepatitis B virus mutants and mechanisms of antiviral resistance of hepatitis B and C.

Theo Heller, M.D., (theller@nih.gov): The virology of acute hepatitis C, utilizing a model of hepatitis C virion production to elucidate the biology of virion assembly and release, and to explore novel therapeutics.

NIMH
National Institute of Mental Health

Postdoctoral Position in Psychology or Psychiatry Mood and Anxiety Disorders Research Program

The Section of Developmental Genetic Epidemiology in the Mood and Anxiety Disorders Program at the National Institute of Mental Health is recruiting a postdoctoral fellow in experimental psychology, biological psychology/psychiatry, clinical psychology, neuro-psychology/psychiatry, or related field. The focus of the section is genetic epidemiologic and community studies, particularly family and high-risk studies of the correlates and risk factors for the development of mood and anxiety disorders. The candidate must have a Ph.D. in psychology or an M.D. with psychiatry residency, and some research experience is preferred. Preference will be given to candidates with a background and interest in the fundamentals of stress, the autonomic nervous system, and/or reproductive endocrinology/hormones. Applicants should send a curriculum vitae, statement of research interests, and three letters of reference to **Dr. Kathleen R. Merikangas, Chair Search Committee, National Institute of Mental Health, 35 Convent Drive, Bldg 35 Room 1A201, MSC-2370, Bethesda, MD 20892-3720.**

The NIH Director's Wednesday Afternoon Lecture Series

Biomedical scientists around the world are invited to join us online to hear leading investigators present their latest results to the NIH Intramural Research community. Lectures may be viewed live at 3:00 p.m., EST (20:00 GMT) on Wednesdays, from September through June. Live webcasts can be viewed under "Today's Events" at: <http://videocast.nih.gov/>

The current schedule of lectures is available at: <http://www1.od.nih.gov/wals/schedule.htm>

Upcoming Lectures:

- September 7: Adrian Krainer, Cold Spring Harbor Laboratories, Alternative Splicing in Health and Disease
- September 14: Solomon Snyder, Johns Hopkins University, Messenger Molecules of Life and Death
- September 21: Amitai Etzioni, George Washington University, How Societies Reach New Shared Moral Understandings
- September 28: Martin Heisenberg, University of Wurzburg, Germany, Mapping Memory Traces in the Fly Brain

The lecture series has archived more than 240 lectures since 1998. Archived lectures can be viewed under "Wednesday Afternoon Lectures" at: <http://videocast.nih.gov/PastEvents.asp>

United States
National Institute of Diabetes & Digestive & Kidney Diseases
of the National Institutes of Health

Research Opportunity at the NIH, DHHS
DIRECTOR, OBESITY CLINICAL RESEARCH CENTER AND CHIEF, DIABETES BRANCH, NIDDK

The Intramural Research Program (IRP) of NIDDK invites applications for the combined position of Chief of the Diabetes Branch and Director of a newly established, NIH-wide initiative in patient-oriented research in obesity ("Obesity Clinical Research Center" – OCRC). The Diabetes Branch, NIDDK conducts basic, translational and clinical research in the areas of diabetes mellitus and obesity. The Chief is responsible for all activities of the Branch, in particular, for integrating the research programs of the several senior investigators and the career development of junior investigators. The goal of the OCRC, which will involve researchers from all Institutes and Centers within the NIH IRP, is to generate knowledge of the pathophysiology, prevention and treatment of obesity and its multisystem co-morbidities, especially type 2 diabetes mellitus. The approach is: 1) to create a center in which to conduct state-of-the-art, patient-oriented obesity research, including metabolic analysis and imaging capabilities, that would support IRP scientists and serve as a magnet facility to foster collaborations with extramural researchers; and 2) to foster multidisciplinary approaches to obesity research, including metabolism, endocrinology, nutrition, gastroenterology, hepatology, imaging, genetics and behavioral sciences.

Priority will be given to applicants at the Professor or Associate Professor level in clinical departments of traditional academic medical centers, or in equivalent positions. The applicant must have a proven record of accomplishments, including evidence of significant, competitively obtained funding for extramural investigators. The appointment will be as a tenured Principal Investigator within NIDDK. The successful candidate will be expected to coordinate the multidisciplinary research proposed for the OCRC and the Diabetes Branch. The position offers unparalleled opportunity to lead a state of the art program in diabetes/obesity research. Salary and benefits are commensurate with the experience of the applicant.

The Diabetes Branch laboratories are in the Warren G. Magnuson Clinical Center and the OCRC Patient Care Unit is a self-contained, metabolic unit located in the new Mark O. Hatfield Clinical Research Center, which are contiguous on the main intramural campus of the NIH in Bethesda, Maryland, a suburb of Washington, D.C.

Interested applicants should send a Curriculum Vitae and list of publications, copies of three major publications, a summary of research accomplishments, a plan for future research and three letters of recommendation to **Dr. James E. Balow, Chair, Search Committee, c/o Ms. Giulia Verzariu, Office of the Scientific Director, NIDDK, Building 10, Room 9N222, NIH, Bethesda, MD 20892.**

Closing Date: October 1, 2005

Department of Health and Human Services
National Institutes of Health
National Institute of Diabetes and Digestive and Kidney Diseases
Equal Opportunity Employers

Department of Micro and Nanotechnology

Technical University of Denmark

Open positions at DTU, MIC - Dept. of Micro and Nanotechnology

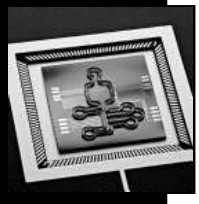
Nano

1 Postdoctoral Position in Nanosystems Fabrication

MIC invites candidates with a PhD degree in physics or engineering to apply for a postdoctoral position in the NanoSystemsEngineering section. We are looking for an experimentalist with experience in nanofabrication (especially e-beam writing) and design of NanoSystems.

Application deadline: 1st October 2005.
Contact: Anja Boisen, email: ab@mic.dtu.dk

Bio



2 Postdoctoral and 2 PhD Positions in Microfabricated Lab-on-a-Chip (Bio-MEMS) for Biotechnological Applications

As part of its interdisciplinary research program, MIC invites candidates with a master or PhD degree in physics, material science, engineering, chemistry or molecular biology to apply for PhD or postdoctoral positions in microfabricated Lab-on-a-Chip (Bio-MEMS) for biotechnological applications. Knowledge of design and fabrication of microsystems, optical detection methods, cleanroom fabrication, and PCR, DNA arrays and molecular biology methods for mutation detection is desirable.

Application deadline: 15th September 2005
Contact: Anders Wolff, email: aw@mic.dtu.dk

1 Postdoctoral Position in Development of Magnetic Bead Sensor for Biodiagnostics in Microfabricated Lab-on-a-Chip

As part of its interdisciplinary research program, MIC invites candidates with a PhD degree in physics, material science, chemistry or engineering to apply for a postdoctoral position for the development of magnetic micro/nano bead sensors for implementation in a lab-on-a-chip system. Knowledge of magnetic materials, sensor design, thin film deposition and characterization is desirable.

Application deadline: 1st October
Contact: Aric Menon, email: am@mic.dtu.dk

Micro

1 Postdoctoral Position in Microsystem Packaging

MIC invites candidates with a PhD degree in physics or engineering to apply for a postdoctoral position in the MEMS section. The project, affiliated with the Applied Sensors group, concerns development of microsystem packaging for multi-sensors. One challenging example is packaging of a MEMS based sensor to be used for fisheries research, where data storage tags are mounted on the back of fish providing valuable information about the fish behaviour.

Application deadline: 1st October 2005
Contact: Erik V. Thomsen, email: evt@mic.dtu.dk

About MIC

MIC represents a cross-disciplinary research environment where micro and nanotechnology are applied to a wide range of scientific disciplines, e.g. mechanics, optics, chemistry, medical technologies, and biotechnology. MIC has access to a state-of-the-art cleanroom, chemical laboratories, biochemical laboratories, and optical laboratories. MIC's vision is to ensure the establishment of a micro and nanotechnology industry in Denmark. Therefore MIC's mission comprises three distinct activities: Scientific research in micro and nanotechnology, education of scientists and engineers in micro and nanotechnology and transfer of scientific knowledge to private industry. MIC employs 90 scientists and engineers among which 45 PhD students. The vast majority of our scientific projects are conducted in collaboration with Danish industry. MIC offers a comprehensive course plan with theoretical and experimental courses that allows students with various scientific backgrounds to learn about the different facets of micro and nanotechnology. In the past decade MIC has generated 10 start-up and spin-off companies. Several of these companies are expected to launch their first micro and nanotechnology-based products in 2005.
For application, please read the full job ad at ***www.mic.dtu.dk*** *and please specify where you saw this job ad*

DEAN

The University of Texas School of Health Information Sciences at Houston

The University of Texas Health Science Center at Houston announces the search for the Dean of the School of Health Information Sciences (SHIS). SHIS is one of six schools of the UT Health Science Center at Houston. The position offers exceptional opportunities for leadership in a comprehensive academic health science center located in the Texas Medical Center.

Applicants for this position must hold a Doctoral degree, have a minimum of ten years of university teaching experience and possess the following attributes:

- A national and international reputation as a researcher in the biomedical and computational sciences.
- Active in the scientific community, as may be evident through membership in or service to national organizations such as grant review boards, academic and professional societies, and various honor academies.
- An understanding of Clinical Informatics, Computational Biomedicine, and Health Science Education Technology and Research.
- An educator with an established record in higher education.
- Demonstrated management and administrative experience in academic institutions.

Nominations and curricula vitae should be forwarded to:
Chair of the School of Health Information Sciences
Dean Search Committee,
The University of Texas Health Science Center at Houston,
Attn: Annette M. Collins
7000 Fannin, Suite 150
Houston, Texas 77030

Or by e-mail to: Annette.M.Collins@uth.tmc.edu

Applications and nominations will be accepted until the position is filled.

THE UNIVERSITY *of* TEXAS
HEALTH SCIENCE CENTER AT HOUSTON

The University of Texas is an Equal Opportunity, Affirmative Action Employer. Minorities and women are strongly encouraged to apply. This is a security-sensitive position and thereby subject to Texas Education code 51.215.

Open track opportunities for Processing Physicist at the University of Nebraska Medical Center

The department of Radiology seeks exceptional scientists, at the post-doctoral or assistant professor level, to join a well-established program investigating the means to improve disease diagnosis and therapeutic monitoring utilizing magnetic resonance imaging (MRI), spectroscopic imaging (MRSI) and single photon emission computed tomography (SPECT). Expertise in image processing, visualization, and database software development are required.

Digital histology and confocal microscopy are joined in an interdisciplinary research program with the MRI, MRSI, and SPECT studies. Translation of rodent imaging to human clinical studies are proposed. Experience in IDL, Matlab, and C++ programming, Unix system administration and/or Bruker MRI system pulse programming are applicable. The development of an independent research program with competitive funding and research publications are required.

Opportunities for collaborations with experimental neuroscience (**http://www.unmc.edu/CNND**), cancer (**http://www.unmc.edu/cancercenter**), bioinformatics (**http://nicls.unmc.edu/news_view.cfm?Page-ID=7**), and clinical neurosciences (**http://www.unmc.edu/neurologicalsciences/**) research are available. Competitive salary is commensurate with experience for this full time position. Please email or send a cover letter, statement of research interests, CV and three letters of reference to:

Michael Boska, Ph.D.
Director, Radiology Research and Associate Professor of Radiology
Department of Radiology
University of Nebraska Medical Center
981045 Nebraska Medical Center
Omaha, NE 68198
Email: **mboska@unmc.edu**

The University of Nebraska is an Equal Opportunity/ Affirmative Action Employer. M/F/H/V.

Faculty Position

The Molecular Biology Program of the Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center (www.ski.edu), has initiated a faculty search at the Assistant Member level (equivalent to Assistant Professor). We are interested in outstanding individuals who have demonstrated records of significant accomplishment and the potential to make noteworthy contributions to the biological sciences as independent investigators. Successful applicants will have research interests that move the Program into exciting new areas that complement and enhance our existing strengths in the areas of maintenance of genomic integrity, regulation of the cell cycle, and regulation of gene expression. Faculty will be eligible to hold appointments in the newly established Gerstner Graduate School of Biomedical Sciences, as well as the Weill Graduate School of Medical Sciences of Cornell University.

Candidates should e-mail their application in PDF format to **molbio@mskcc.org** by November 1, 2005. The application should include a Curriculum Vitae, a description of past research, a description of proposed research, and representative publications. Candidates should arrange to have three letters of reference sent by mail to **Dr. Kenneth Marians, c/o Steven Cappiello, Box 193, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021.** The letters should arrive by November 1, 2005. The application may be sent by regular mail, but in that case should include a CD containing the application in PDF format. Inquiries may be sent to **Mr. Cappiello at molbio@mskcc.org or to Dr. Kenneth Marians, Chair, Molecular Biology Program, kmarians@sloankettering.edu.**

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Smoke-free environment.

Memorial Sloan-Kettering Cancer Center
The Best Cancer Care. Anywhere.
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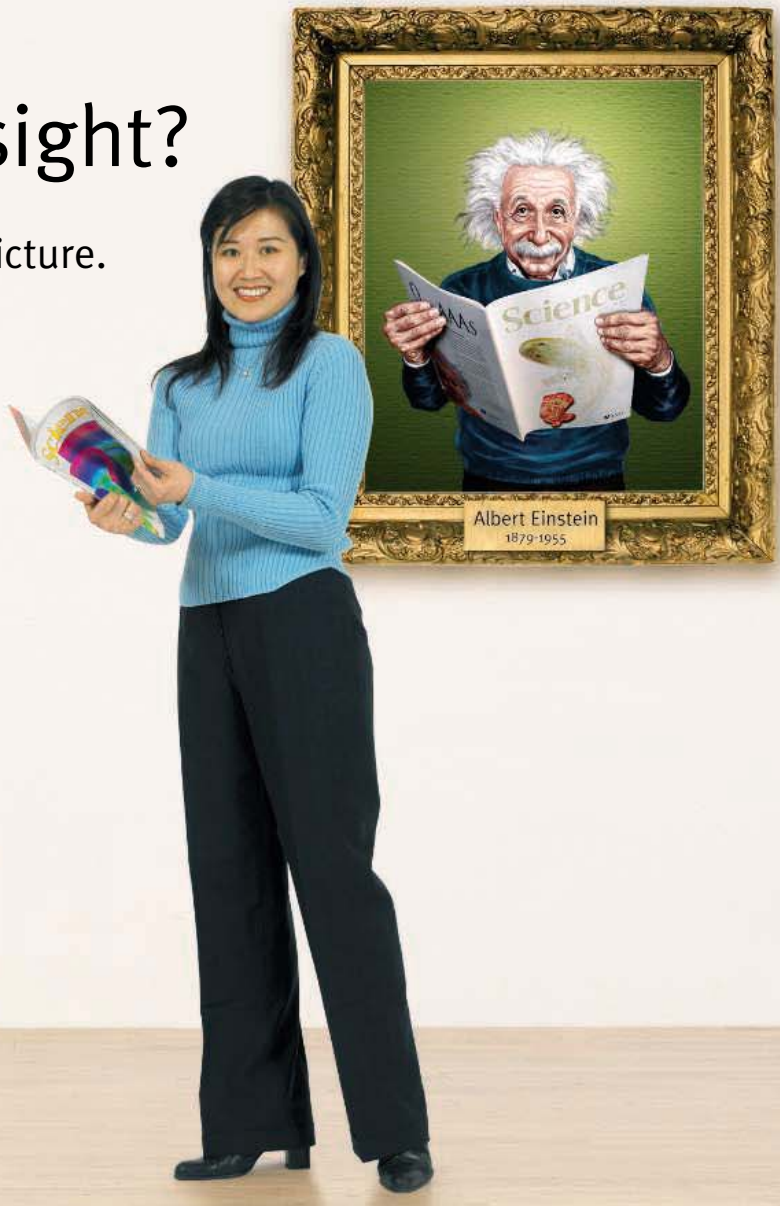

Your career is too important to leave to chance. So to find the right job or get career advice, turn to the experts. At ScienceCareers.org we know science. And we are committed to helping take your career forward. Our knowledge is firmly founded on the expertise of *Science*, the premier scientific journal, and the long experience of AAAS in advancing science around the world. Put yourself in the picture with the experts in science. Visit www.ScienceCareers.org.

ScienceCareers.org
We know science AAAS

Department of Health and Human Services
National Institutes of Health
National Heart, Lung, and Blood Institute

PHYSICIAN OR SCIENCE ADMINISTRATOR
Respiratory Sciences
($62,886 to $114,882)

Airways Diseases Program, Division of Lung Diseases, is seeking an experienced pulmonary researcher, expert in cell/molecular biology, physiology, biochemistry, or clinical trials to provide scientific support in the management and development of our extramural grant program in chronic obstructive pulmonary disease.

The candidate selected will provide leadership for established national programs of airways disease and creativity in the development of new programs in his/her area of expertise. While the primary interest will be chronic obstructive pulmonary disease, other program areas to which the candidate may contribute include asthma, cystic fibrosis, genetics, respiratory neurobiology and sleep.

Candidates must have an M.D., Ph.D. or equivalent, be an experienced, creative research scientist, have demonstrated ability to work effectively with others, and desire to pursue a research administrative non-laboratory career track. The applicant must demonstrate research experience in at least one of the following: cellular and molecular biology; immunology, genetics; pharmacology, clinical trials and respiratory diseases research. The required knowledge, skills, and abilities (KSAs) are: **(1)** Scientific knowledge and research expertise in any of the following: cellular and molecular biology; immunology, genetics; pharmacology, clinical trials and respiratory diseases research; **(2)** Ability to initiate and manage an independent scientific research project; **(3)** Ability to communicate orally and in writing with co-workers and those outside the organization; **(4)** Ability to lead individuals/groups to accomplish a project.

Benefits: Appointment will be made at GS-12/13/14 grade level depending on qualifications. A Physician Comparability Allowance may be paid up to $30,000 per year. In addition, a recruitment bonus may also be considered. Excellent health, life, investment, and personal leave benefits.

Selective Factors: U.S. citizenship is required. For the basic qualification requirements, refer to the NIH guidance for Health Scientist Administrators or Medical Officers. **http://www.nhlbi.nih.gov/about/jobs/hsaguide.htm**
www.opm.gov/qualifications/SEC-IV/B/GS0600/0602.HTM

How to Apply: Position requirements and detailed application procedures are provided in two separate vacancy announcements. Please access www.usajobs.opm.gov and refer to **NHLBI-05-92506** for Science Administrators and **NHLBI-05-92507** for Physicians. Submit a resume, c.v./bibliography or other format to: Kathryn Osbourn, Human Resources Specialist, Two Democracy Plaza, Suite 901, 6707 Democracy Blvd., Bethesda, MD 20817-2157. All applications must be postmarked by the closing date **10/17/2005**. For additional information contact Kathryn Osbourn at (301) 402-8031.

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THE UNIVERSITY OF CALIFORNIA, SAN DIEGO
DIVISION OF BIOLOGICAL SCIENCES
www-biology.ucsd.edu

The Division of Biological Sciences at UCSD has a large faculty spanning many areas of biology and one of the largest and most diverse graduate programs in the country. We invite applications for a faculty position in the

NEUROBIOLOGY SECTION

We plan to make an appointment at the Assistant, Associate, or Full Professor levels in several areas of neurobiology, including systems, computational, behavioral, and developmental neurobiology. Candidates must have demonstrated ability to develop a rigorous research program. All candidates must have Ph.D. or equivalent degree and a commitment to teaching at the undergraduate and graduate levels. The level of appointment will be commensurate with qualifications and experience with salary based on UC pay scale.

Applications received by **October 1, 2005** will be assured of consideration. Send curriculum vitae, complete list of publications, brief description of research interests and professional goals, and the names of at least three references with addresses, emails, phone and fax numbers to: **Neurobiology Search Committee, Division of Biological Sciences 0357-B, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0357**.

UCSD is an Equal Opportunity-Affirmative Action Employer with a strong institutional commitment to the achievement of diversity among its faculty and staff.

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Sanger Postdoctoral Fellowship

The Wellcome Trust Sanger Institute is at the forefront of experimental and computational genome research. We are recognised leaders in genome sequencing, high throughput systems, informatics and analysis of gene function using genetic approaches in a variety of model organisms and humans.

Applications are invited for **Postdoctoral Research Fellowships** within the Sanger Faculty offering state-of-the-art facilities. Successful candidates will be awarded a fellowship with a competitive starting salary plus excellent benefits. This post is initially offered for three years after which it is expected that the candidate will seek independent support. We are particularly interested in hearing from candidates who have completed their PhD within the last year.

To apply please complete the on-line application form at **http://www.sanger.ac.uk/careers/postdoc/** and attach a list of your publications, a one page description summarising your research accomplishments to date and a two page synopsis of the proposed research programme. You are also expected to provide three letters of reference at this point.

If you are interested you **must** in the first instance contact a current member of the Sanger Institute Faculty **(http://www.sanger.ac.uk/Teams/faculty/)** to discuss potential projects.

The closing date for applications is 16 Sep 2005. Applications received after this date, or those which do not conform to the above criteria will not be accepted.

Questions regarding the process can be emailed to **humanresources@sanger.ac.uk** or by contacting Human Resources on telephone number: 01223 494882

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RWTH RHEINISCH-WESTFÄLISCHE TECHNISCHE HOCHSCHULE AACHEN

IZKF MAT.

RWTH Aachen University, University Hospital Aachen, IZKF "BIOMAT.", Germany

The Interdisciplinary Center for Clinical Research IZKF "BIOMAT." within the faculty of Medicine at the RWTH Aachen University is seeking an enterprising colleague interested in the basic development and clinical applications of neurovascular magnetic resonance imaging (MRI) as a

Post-doctoral Fellowship in Clinical MRI-Physics

The position will be closely linked to the MR-physics group of the Department of Radiology. It involves research on developing techniques for rapid structural and functional brain MRI at high magnetic field strengths including new contrast mechanisms and parallel imaging strategies. Ultimately this work will be applied to non-invasively diagnosing neurological and neuropsychiatric disorders.

The position would be well suited for a scientist with strong initiative and excellent communication skills. Candidates must have a PhD in physics, electrical engineering, computer science or other related fields. Prior experience in clinical MR research or engineering and expertise in MR pulse sequence development is required. Experience with parallel and functional MRI is desirable, preferably but not necessarily on a Philips MR scanner.

Opportunities will exist to collaborate with researchers in the Radiology, Neuroradiology, Neurology and Psychiatry department. Interdisciplinary collaboration with other faculties of the RWTH Aachen is highly encouraged. State-of-the-art research MR instruments are available including 32-channel 3.0 Tesla and 8-channel 1.5T Philips Achieva whole body systems dedicated to research and several clinical 1.5 Tesla Philips scanners.

The appointment will be initially for a period of 3 years at the Senior Research Fellow level with the option to expand the appointment. The position includes a competitive salary following the German civil service pay guidelines.

The RWTH Aachen encourages the employment of women. Qualified women are therefore especially invited to apply.

Handicapped applicants are favourably considered in the case of equal qualification.

Interested candidates should send curriculum vitae and cover letter (by email or postal mail) to:

Armin Thron, MD, Professor of Neuroradiology
Thoralf Niendorf, PhD, Professor of Experimental Magnetic Resonance Imaging
Aachen University, University Hospital, Pauwelsstrasse 30, 52057 Aachen, Germany

Phone: ++49 241 80 80295, mail to: niendorf@rad.rwth-aachen.de; athron@ukaachen.de

Faculty Position In Cell Biology

The Department of Molecular Biology & Genetics seeks a tenure-track Assistant Professor of Cell Biology studying the functional organization of cells or tissues. Applicants including focused genomic approaches to questions in cell biology are especially encouraged to apply. The position is part of a campus-wide expansion in cell biology, which includes a new Institute of Molecular and Cell Biology. The successful candidate will be expected to establish a vigorous independent research program and participate in the teaching of cell biology. The department includes faculty with research programs in cell biology, developmental biology, genetics, molecular biology, comparative and population genomics, structural biology, and biochemistry (**http://www.mbg.cornell.edu**).

Candidates should submit a curriculum vitae, a description of research plans and teaching interests, plus copies of two papers, as a single PDF file (max. 5MB) to: **Tony Bretscher, c/o RLL2@cornell.edu** and arrange for three letters of recommendation to be sent electronically to **RLL2@cornell.edu** and in hard copy to:

Tony Bretscher
Cell Biology Search Committee
107 Biotechnology Bldg.
Cornell University
Ithaca, NY 14853

Cornell University

The committee will begin reviewing applications on October 15, 2005.

Cornell University is an Affirmative Action/ Equal Opportunity, Employer and Educator

http://chronicle.com/jobs/profiles/2377.htm

The University of Texas at Austin

Eukaryotic Molecular Biology Positions

The Institute for Cellular and Molecular Biology

The Institute for Cellular and Molecular Biology invites applications for tenure-track/tenured positions in eukaryotic molecular biology. Academic appointments at the level of Assistant, Associate, or Full Professor will be in an appropriate academic unit in the College of Natural Sciences. Candidates should have an outstanding record of research productivity and a research plan that utilizes molecular and biochemical approaches to address important problems in eukaryotic molecular biology. Areas of particular interest include but are not limited to chromatin structure, regulation of gene expression, RNA interference, DNA damage responses, and cell cycle control.

Building on a strong existing faculty, the Institute has recruited more than 30 new faculty members over the past seven years (see www.icmb.utexas.edu). In addition to an interactive and interdisciplinary research environment, the Institute provides administrative and financial support for the Graduate Program in Cell and Molecular Biology and state-of-the-art core facilities including mass spectrometry, electron and confocal microscopy, DNA microarrays, robotics, and mouse genetic engineering.

Austin is located in the Texas hill country and is widely recognized as one of America's most beautiful and livable cities.

Please send a single PDF file containing your curriculum vitae, summary of research interests, and names of three references before November 1, 2005 to **icmbfacultysearch@biosci.utexas.edu**. In addition, send a hard copy of the same addressed to the co-chairs of the search committee:

Dr. Tanya Paull and Dr. Jon Huibregtse
Eukaryotic Molecular Biology Search
Institute for Cellular and Molecular Biology
The University of Texas at Austin
1 University Station A4800
Austin, TX 78712-0159

Homepage • http://www.icmb.utexas.edu

The University of Texas at Austin is an Equal Opportunity Employer. Qualified women and minorities are encouraged to apply; a background check will be conducted on applicant selected.

INTERNATIONAL CAREERS REPORT

Recruit qualified scientists by advertising in *Science*'s Careers feature on

GERMANY

Issue date ..... **7 October 2005**
Booking deadline ..... **20 September 2005**

To profile your organisation or to place a recruitment advertisement, please contact Christina Harrison.

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Fax ..... +44 (0) 1223 326532
E-mail ..... charrison@science-int.co.uk

ScienceCareers.org
We know science AAAS

Florida State University
National High Magnetic Field Laboratory
Senior Faculty Professor

The Department of Mechanical Engineering at the Florida A&M/Florida State University College of Engineering seeks a senior scientist with superb research and teaching credentials at the Full Professor level. The successful candidate will develop and lead a materials science research program that builds on existing strengths of the FAMU/FSU College of Engineering and the National High Magnetic Field Laboratory's (NHMFL) mission to develop materials relevant to the advance of its state-of-the-art high magnetic field facilities. In addition to serving as Full Professor, the successful candidate will serve as Chief Materials Scientist of the NHMFL, with duties and responsibilities to be developed in consultation with the Director of the NHMFL.

Minimum qualification includes a PhD in materials science, materials engineering, materials physics or related field and ten years professional experience in materials research and teaching. Leadership skills, international scientific reputation, scientific vision and a demonstrated ability to raise research funding are requirements. Salary will be commensurate with experience.

Interested candidates will need to apply to Florida State University at **https://jobs.fsu.edu** and reference **Job Requisition #2895**. Please attach your curriculum vitae, cover letter describing your experience, and names and contact information of three references. For additional information, please contact: **Ms. Bettina Roberson, National High Magnetic Field Laboratory, Florida State University, 1800 E. Paul Dirac Drive, Tallahassee, FL 32310-2740, 850-644-0855**. Additional information may be attached and emailed to **roberson@magnet.fsu.edu**.

The Florida State University is an Equal Opportunity, Affirmative Action Employer, committed to diversity in hiring, and a Public Records Agency.

University of Pennsylvania - School of Medicine
Department of Medicine - Division of Medical Genetics
Gene Therapy Program

CHIEF SCIENTIFIC OPERATING OFFICER

The Gene Therapy Program at the University of Pennsylvania is looking for a scientist to direct the operations of a large multi-disciplinary research and development program. The organization is housed at Penn and is supported by a diverse array of grants and contracts from the federal government, foundations and the biopharmaceutical industry. The central theme of the research is the development of gene transfer technology and its use in vaccines and gene therapy.

A vibrant discovery effort forms the foundation for the research and is supported by five core laboratories: Vector, Cell Morphology, Quality Control, Immunology, and Animal Models. The leadership team includes (1) Director of the Gene Therapy Program who has overall responsibility for the Program and who directs the science; (2) Chief Financial Officer (CFO) who is responsible for human resources and all financial aspects of the program; and (3) Chief Operating Officer (COO) which is the focus of this recruitment. The CFO and COO report to the Director.

We are looking for an outstanding senior individual who will oversee operations of the Discovery Research and Core laboratories, management of the Animal Models Program, Project Management, and Technology Transfer.

Successful candidate will have a PhD and/or MD. Academic or industry experience necessary. Proven administrative, scientific and management skills required. Experience in intellectual property and laboratory animal research preferred.

Candidates should send a letter of interest and curriculum vitae to: **CSOO Search, Translational Research Laboratory, Gene Therapy Program, 125 S. 31st Street – Suite 2000, Philadelphia PA 19104-3403** or email **GTP@mail.med.upenn.edu**.

The University of Pennsylvania is an Affirmative Action/ Equal Opportunity Employer.

ASSISTANT-ASSOCIATE-FULL PROFESSOR POSITION

The H. Lee Moffitt Cancer Center and Research Institute, an NCI-designated Comprehensive Cancer Center, and the Department of Interdisciplinary Oncology at the University of South Florida College of Medicine, are seeking candidates for Assistant, Associate, or Full Professor level individuals to participate in the Drug Discovery Program. Successful candidates must possess a Ph.D. or M.D. degree and a demonstrated potential for extramural funding. We are looking for individuals with a proven consistent publication record to complement current existing interests in our program including, but not limited to, the broad areas of signal transduction, gene regulation, cell cycle, apoptosis, angiogenesis and proteomics.

Assistant Professor must have a Ph.D. or M.D. with postdoctoral experience in any areas of molecular biology, biochemistry, or cell biology, and high quality publications in peer-reviewed journals. The Associate/Full Professor must have a Ph.D. or M.D. with a proven track record of independent research and demonstrated sustained extramural funding. In addition, the Associate Professor rank requires at least five years experience with continuing and productive service as an Assistant Professor. The Professor rank requires documentation of national recognition, leadership ability and at least five years experience with continuing and productive service as an Associate Professor. Application review begins August 1, 2005.

Please refer to position no. DIO0514. Interested candidates should send curriculum vitae and a brief statement of major academic interests, and request at least three letters of recommendations sent to Said M. Sebti, Ph.D., Associate Director, Moffitt Research Institute, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, FL 33612.

H. LEE MOFFITT Cancer Center & Research Institute

The End Of Cancer Begins Here.

A National Cancer Institute Comprehensive Cancer Center
At the University of South Florida

USF University of South Florida College of Medicine

The University of South Florida is an EO/AA/EA institution. For disability accommodations, contact Kathy Jordan 813-632-1451 a minimum of five working days in advance. According to Florida law, applications and meetings regarding them are open to the public.

www.moffitt.usf.edu

POSITIONS OPEN

STANFORD UNIVERSITY DEPARTMENT OF MOLECULAR PHARMACOLOGY

The Department of Molecular Pharmacology at Stanford University School of Medicine invites applications for two tenure-track or tenured positions (MP1 and MP2) at the **ASSISTANT** or **ASSOCIATE PROFESSOR** levels. For position MP1, candidates whose research interests lie at the interface of biomedical and physical sciences (e.g., chemical biology, quantitative biology, systems biology, and/or combinatorial biochemistry) are particularly encouraged to apply. For position MP2, outstanding applicants in any area of signal transduction or cellular regulation are welcome. Stanford offers an outstanding environment for creative interdisciplinary biomedical research. Rank and salary are dependent on the candidate's qualifications. The predominant criterion for tenure-track University appointment is a major commitment to research and teaching.

Candidates should have a Ph.D. and/or M.D. degree and postdoctoral research experience. Candidates should reference the appropriate position code (MP1 or MP2) and send curriculum vitae, a description of future research plans, and the names of three references by October 15, 2005, to:

Daria Mochly-Rosen, Ph.D.
c/o Jean Kavanagh, FAA
Department of Molecular Pharmacology
269 Campus Drive, CCSR Building
Room 3145A
Stanford University
School of Medicine
Stanford, CA 94305-5174

Stanford University is an Equal Opportunity/Affirmative Action Employer.

BIOPHYSICAL CHEMISTRY
Dartmouth College

Applications are invited for a faculty position at the **ASSISTANT PROFESSOR** level starting July 2006. The Chemistry Department seeks an individual who will establish a nationally recognized research program in experimental biophysical chemistry at Dartmouth, and who will excel at teaching in our undergraduate and Ph.D. curriculum. Preference will be given to individuals who investigate structure-function relationships of biological macromolecules using physical techniques complementing existing strength in X-ray crystallography. Candidates will be expected to contribute to the teaching of courses in our biophysical chemistry major, graduate courses in their area of research, and introductory chemistry. Applicants should submit curriculum vitae, a description of their research plans, and a brief statement about their teaching interests. Applicants should also arrange to have three letters of recommendation sent on their behalf. All inquiries and applications will be treated confidentially. Application materials should be sent to: **Chair, Biophysical Chemist Search Committee, Department of Chemistry, 6128 Burke Laboratory, Dartmouth College, Hanover, NH 03755-3564.** The Committee will begin to consider completed applications on October 15, 2005. *With an even distribution of male and female students and over a quarter of the undergraduate student population members of minority groups, Dartmouth is committed to diversity and encourages applications from women and minorities. Dartmouth College is an Equal Opportunity/Affirmative Action Employer.*

The Global Carbon Project, a joint program of the International Human Dimensions Programme, International Geosphere-Biosphere Programme, World Climate Research Programme, and International Programme of Biodiversity (DIVERSITAS) under their Earth System Science Partnership, seeks **EXECUTIVE OFFICER** of its International Project Office in Tsukuba, Japan, at the National Institute for Environmental Studies. See announcement at **website: http://www.globalcarbonproject.org.** Deadline: 1 October 2005. Commence: February 1, 2006.

POSITIONS OPEN

HEAD, DEPARTMENT OF TOXICOLOGY
College of Pharmacy
The University of Louisiana Monroe

Applications are invited from qualified candidates for the position of Head, Department of Toxicology. Applicants must possess a Ph.D. degree in toxicology or closely related field and meet tenure eligibility requirements at the **ASSOCIATE PROFESSOR** or **PROFESSOR** level. Successful candidate will have an outstanding scientific background in toxicology, an established record of grantsmanship, productivity, and the ability to foster interdisciplinary research. In addition, the applicant should possess excellent communication and interpersonal skills, demonstrated leadership to ensure successful operations of an academic department, and the ability to recruit students and faculty to both undergraduate and graduate degree programs in toxicology. Responsibilities include day-to-day operations of a B.S. degree program in toxicology currently enrolling 60-70 majors as well as a very active graduate program with 12-14 M.S. and Ph.D. candidates.

The University of Louisiana Monroe (ULM)'s College of Pharmacy offers the only state-supported Pharm.D. degree program in Louisiana and is the University's flagship program. The College will be moving to its new 132,000 square feet modern state-of-the-art facilities during the 2005-2006 academic year. For more information on ULM and surrounding region visit **websites: http://www.ulm.edu** and **http://www.monroe.org.**

Interested individuals meeting these qualifications should submit an application portfolio that includes: a letter of interest giving a summary of achievements, visionary goals for the Department in research, education, and service; curriculum vitae, copies of three recent publications, and names and contact information for five references to: **Harihara M. Mehendale, Ph.D., Department Head Search Committee, Department of Toxicology, College of Pharmacy, The University of Louisiana Monroe, 700 University Avenue, Monroe, LA 71209. Telephone: 318-342-1691; e-mail: mehendale@ulm.edu.** Electronic submissions are encouraged. Applications must be received by October 3, 2005, but the review of applications will begin immediately and continue until the position is filled. *The University of Louisiana at Monroe is an Equal Opportunity/Affirmative Action Employer.*

MOLECULAR MICROBIOLOGIST

The Department of Biological Sciences, Texas Tech University invites applications for a tenure-track Molecular Microbiologist at the **ASSISTANT PROFESSOR** rank, beginning September 2006. A Ph.D. in microbiology, immunology, or a related field and postdoctoral experience are required. The successful candidate will establish an active, extramurally funded collaborative research program in an area of microbiology utilizing modern molecular approaches. The potential to develop collaborative research initiatives with colleagues in the Department and across the University will be an important consideration in the selection process. Current microbiological research in the Department includes but is not limited to ecology, genetics, host interactions, immunology, mycology, physiology, and virology. The new Experimental Sciences Building adjacent to Biology provides support facilities for biotechnology, genomics, imaging, and bioinformatics. Plant growth, animal care, and greenhouse facilities are available. Teaching duties would include general microbiology along with an advanced and graduate specialty course. Applicants should submit a cover letter, curriculum vitae, and descriptions of research goals and of teaching interests and philosophy, and should arrange for three letters of recommendation to be forwarded. Applications can be submitted at **website: http://jobs.texastech.edu,** by **e-mail: lanita.ladd@ttu.edu,** or mail to: **Microbiologist Search, c/o John Zak, Chair, Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131.** Applications should be received by October 14, 2005. Visit our **website: http://www.biol.ttu.edu.** *Women and members of underrepresented groups are encouraged to apply.*

POSITIONS OPEN

ACADEMIC POSITIONS

Swarthmore College invites applications for two tenure-track positions in the Department of Chemistry and Biochemistry at the **ASSISTANT PROFESSOR** level. The first position is in inorganic chemistry. Primary teaching responsibilities will center around intermediate and advanced inorganic chemistry as well as general chemistry. The field for the second position is open, but with the expectation that the appointee will assume a leadership role in developing and implementing a junior-level course in the theory and practice of laboratory instrumentation and/or analytical methods. Primary teaching responsibilities will be in this course, which the Department plans to offer beginning in the fall of 2006, as well as in other areas of the curriculum matching the appointee's expertise. For both positions, the Department will look with particular favor upon applicants whose research interests have a biochemical dimension. It is expected that both appointees will conduct active research programs involving Swarthmore undergraduates (median SAT score, approximately 1,450 out of 1,600). The College offers a generous sabbatical policy, and also provides competitive startup funds and internal support for student summer stipends and supplies. Both appointments are scheduled to begin on September 1, 2006. Ph.D. required; postdoctoral experience is preferred. Candidates are requested to submit curriculum vitae, official copies of graduate and undergraduate transcripts, statements of teaching and research goals, and should arrange for a minimum of three letters of recommendation to be sent to: **Professor Paul R. Rablen, Chair, Department of Chemistry and Biochemistry, Swarthmore College, 500 College Avenue, Swarthmore, PA 19081-1397.** Applications must be completed by October 3, 2005, to assure full consideration. *Swarthmore is an Equal Opportunity Employer/Women and members of underrepresented minorities are encouraged to apply.*

GENERAL ADVERTISEMENT

The Department of Chemistry at the University of Michigan invites applications for an anticipated position at the rank of **ASSISTANT PROFESSOR** or **ASSOCIATE PROFESSOR** in any subdiscipline of chemistry with a proposed start date of September 1, 2006. This would be a University-year appointment (nine months academic salary with three months research supported salary). Candidates are expected to develop an internationally recognized program of scholarly research and to excel in teaching at undergraduate and graduate levels. Detailed information regarding the electronic application process and required materials is available online at **website: http://www.chem.lsa.umich.edu/chem/facultyrecruit/.** The position will remain open until filled but preference will be given to applicants who have submitted all requested materials prior to October 15, 2005. Information about the Chemistry Department is available on the **website: http://www.umich.edu/~michchem.** Questions about the applications process should be sent to **e-mail: chemfac05@umich.edu.** *Women and minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual career couples and is a nondiscriminatory, Affirmative Action Employer.*

The Department of Ecology and Evolutionary Biology, Tulane University, invites applications for one tenure-track position in phylogenetic systematics at the level of **ASSISTANT PROFESSOR.** See the **website: http://www.tulane.edu/~ebio/News/positions.htm** for more details. Send curriculum vitae, statements of research and teaching interests, selected publications, and names and addresses of three references to: **Phylogenetic Systematist Search, Department of Ecology and Evolutionary Biology, 310 Dinwiddie Hall, Tulane University, New Orleans, LA 70118-5698.** Review of applications will begin October 14, 2005, and the search will remain open until the position is filled. *Tulane University is an Affirmative Action/Equal Employment Opportunity Employer.*

INORGANIC AND MATERIALS CHEMISTRY

The Department of Chemistry at Emory University announces a search for a tenure-track position in research areas broadly defined by bioinorganic chemistry, catalysis, inorganic materials or nanoscience. The position will strengthen our inorganic/materials chemistry program and/or complement recent growth at the interface of chemistry and biology. Candidates must have a PhD degree in chemistry or related fields. Successful candidates are expected to establish a creative multidisciplinary research program and commit to quality teaching at both the undergraduate and graduate levels. The appointment is expected to be at the rank of **Assistant or Associate professor**. For the Associate professor appointment, applications are welcome from individuals currently at the Assistant or Associate levels with unusually strong records.

Candidates should submit a letter of intent, a curriculum vitae and research plans, and arrange to have three letters of recommendation sent. All materials and correspondence should be sent via email (preferred) with attached Microsoft Word or Adobe Acrobat files to: **chemsearch@learnlink.emory.edu**, or alternatively by regular mail to: Chair, Inorganic Faculty Search Committee, Department of Chemistry, 1515 Dickey Dr., Emory University, Atlanta, GA 30322. For more information about the Department, please visit the website: **http://www.emory.edu/CHEMISTRY**. Application review will begin **October 1, 2005** and will continue until the position is filled.

EMORY UNIVERSITY

Emory University is an Equal Opportunity/ Affirmative Action Employer.

WEILL CORNELL MEDICAL COLLEGE IN QATAR

FACULTY POSITIONS

In a pioneering international initiative, Weill Medical College of Cornell University established the Weill Cornell Medical College in Qatar (WCMC-Q) through a unique partnership with the Qatar Foundation for Education, Science and Community Development. Located in Doha, Qatar, and in its fourth year of operation, Weill Medical College of Cornell University seeks candidates for faculty positions to teach in Doha in:

• Cell Biology • Cell Physiology • Genetics • Molecular Biology • Molecular Pharmacology • Pharmacology • Physiology

Following a two-year Pre-medical Program, the inaugural class has now completed the first year of the traditional four-year education program leading to the Cornell University M.D. degree, which they will receive in May 2008. The medical program at WCMC-Q replicates the admission standards and the innovative problem-based curriculum, which includes, among other things, integrated, multidisciplinary basic science courses that are the hallmark of the Weill Medical College of Cornell University.

Faculty, based in Doha, will be expected to teach their specialty and to contribute to the academic life of the Medical College. This unique program provides the successful applicant with the opportunity to leave his/her mark on a pioneering venture. A state of the art research program, to be housed in WCMC-Q and focused on genetics with an emphasis on diabetes, obesity, hypertension and metabolic bone disease will be initiated within the next year. Teaching and research facilities are situated within a brand new building designed to Cornell specifications and located in Education City in Doha amongst other American universities.

All faculty members at WCMC-Q are appointed by the academic departments at Weill Medical College of Cornell University.

Further details regarding the WCMC-Q program and facilities can be accessed at: www.qatar-med.cornell.edu.

Candidates should have a M.D., Ph.D. or M.D./Ph.D. or equivalent terminal degree. Salary is commensurate with training and experience and is accompanied by an attractive foreign-service benefits package. Applicants should submit a letter of interest outlining their teaching and research experience and curriculum vitae to:

facultyrecruit@qatar-med.cornell.edu

***Please quote *Faculty Search #05-016-sci* on all correspondence**

Weill Medical College of Cornell University is an equal opportunity, affirmative action educator and employer.

The screening of applications will begin immediately and continue until suitable candidates are identified.

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Search a comprehensive list of job postings from these employers on **ScienceCareers.org.** Listings updated three times a week.

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ScienceCareers.org
We know science AAAS

SYRACUSE UNIVERSITY

Department of Biology
Tenure-Track Faculty Position
in Ecology & Evolutionary Biology

The Department of Biology at Syracuse University invites applications for a tenure-track position (Assistant or Associate Professor) to be filled by August 2006. The successful candidate will have (Associate Professor) or will develop (Assistant Professor) a strong, extramurally funded and highly innovative research program in ecology and/or evolutionary biology to join an emerging interdisciplinary research group in biocomplexity. Suitable research programs may include theoretical or empirical studies of molecules, organisms or ecosystems. The successful applicant will complement current research strengths within the department and university related to the evolution and functioning of complex adaptive biological systems in different environments. The Department and the University place a high priority on effective undergraduate and graduate teaching.

The successful candidate will join a highly productive faculty with strong links to other programs at Syracuse University, including engineering, environmental policy, biochemistry and earth sciences. The Syracuse biocomplexity group also has close intellectual ties to more than 60 other faculty at several other universities including the nearby State University of New York Environmental Science and Forestry school (SUNY-ESF) and Cornell University. Collaborations among the faculty in this group would allow successful applicants to explore several new interdisciplinary funding initiatives at NSF, including NSF's Biocomplexity Initiative, Emerging Frontiers, and Biology & Mathematics programs.

The Biology Department is in the midst of an exciting growth period, having hired nine new faculty in the past five years and with construction beginning on a new Life Sciences Building (anticipated move-in, 2008). We anticipate hiring 6-10 more new faculty over the next five years. Specific information about individual Biology faculty research programs may be found on our website:

http://biology.syr.edu/facultyresearch/facultyresearch.html

Applicants should forward a curriculum vitae, a description of past research accomplishments, a clearly focused description of his/her proposed future research goals and a statement of teaching interests. We also request that applicants have at least three letters of reference sent. Please include the name, address, phone number and e-mail address of each of your references. We invite applicants to submit materials electronically as a single PDF file to: biosearch@cas.syr.edu. The position will be open until filled, but to be assured your application receives full consideration, we urge that you arrange to have all necessary materials to us by September 16, 2005.

Applications and reference letters should be addressed to: **Scott Pitnick, Chair of Eco-Evo Faculty Search, Department of Biology, 130 College Place, Syracuse University, Syracuse, NY 13244**

Syracuse University is an Affirmative Action/Equal Opportunity Employer.

POSITIONS OPEN

FACULTY POSITIONS, CHEMISTRY
University of Florida

The Department of Chemistry at the University of Florida announces a search for tenure-track faculty members to begin in fall 2006. Candidates with research interests in the areas of macromolecular chemistry or in physical biochemistry are invited to apply. For the macromolecular/polymer chemistry area, candidates at the **ASSISTANT** or beginning **ASSOCIATE PROFESSOR** levels will be considered. For the physical biochemistry area (experimental physical, structural, or analytical methods applied to biochemistry), candidates at the Assistant Professor level will be considered. In addition to contributing to the research, teaching, and service missions of the Department of Chemistry, we anticipate campus-wide interactions in interdisciplinary programs with departments in the Colleges of Liberal Arts and Sciences, Medicine, and Engineering, along with other University-based Centers and Institutes. Applicants should submit curriculum vitae, description of their research plans (specify either macromolecular or physical biochemistry research area in the cover letter), graduate/undergraduate teaching interests, and arrange to have three letters of recommendation sent on their behalf to: **The Faculty Search Committee, Department of Chemistry, Box 117200, University of Florida, Gainesville, FL 32611-7200** on or before October 7, 2005. *The University of Florida is an Equal Opportunity Institution and welcomes nominations and applications from women and minority group candidates.*

EVOLUTIONARY BIOLOGY FACULTY POSITION
UCLA Department of Ecology and Evolutionary Biology

The Department of Ecology and Evolutionary Biology at UCLA invites applications for an open rank, tenure-track faculty position in evolutionary biology, broadly defined. The expected start date is September 2006. Candidate must have a Ph.D.; postdoctoral experience is desired. Salary is commensurate with education and experience. Successful candidates are expected to maintain a rigorous research program, and to contribute to undergraduate and graduate teaching. UCLA has outstanding academic support for faculty, including access to the University of California Natural Reserve System, a campus-wide Institute of Pure and Applied Mathematics, several departments with computational and evolutionary biology interests, and attractive startup packages. Submit curriculum vitae, statements of research and teaching interests, and names and addresses of three references online to **website: http://www.eeb.ucla.edu/Evolutionist.** Please contact: **Charles Taylor (e-mail: taylor@biology.ucla.edu)** for additional information. Reviews of applications will begin September 30, 2005.

The University of California is an Equal Opportunity Employer committed to excellence through diversity.

PHARMACOLOGY FACULTY POSITION
Touro University-California

Touro University-California, located in the northern San Francisco Bay Area, seeks a full-time pharmacologist, open rank. Teaching duties include participating in team-taught, integrated basic medical science courses in pharmacology/neurosciences. Research space and initiation support are available, and candidates are expected to develop a rigorous research program. This position begins 1 January 2006. Review of applications begins immediately and continues until the position is filled. Please submit curriculum vitae, statement of research interests, and contact information for three references to: **Dr. Jean-Marc Schwarz, Touro University College of Osteopathic Medicine, 1310 Johnson Lane, Vallejo, CA 94592,** or via **e-mail: jschwarz@admin2.touro.edu.**

Touro University-California is an Equal Opportunity/Affirmative Action Employer.

ASSISTANT PROFESSOR (NONTENURE TRACK)

The Institute of Human Virology, University of Maryland Biotechnology Institute (UMBI), has an immediate opening for a nontenure-track faculty to study the structure and function relationships for anti-tumor, anti-HIV, and antibacterial peptides. Experimental approaches used in this study include, but are not limited to, (1) peptide/protein synthesis and chemical modification, (2) biochemical and biophysical as well as structural characterization. The appointee is also expected to apply for extramural funding to support his or her own independent research complementary to the existing scientific programs of the Institute.

Qualified candidates must have a Ph.D. in chemistry/biochemistry or related field, with at least five years postdoctoral experience and an outstanding publication record in peptide and protein chemistry. Salary is commensurate with experience and qualifications. Please send a letter of application (referencing position #300608), current resume, and names and telephone numbers of three references to the following address: **Dr. Wuyuan Lu, Institute of Human Virology, 725 West Lombard Street, Baltimore, MD 21201** or **e-mail: luw@umbi.umd.edu.** Review of applications will begin August 26, 2005, and continue until a suitable candidate is selected. *UMBI is committed to Affirmative Action and Equal Opportunity Employment. As required by the 1986 Immigration Act, applicants should be prepared to present acceptable documentation showing their identities, their U.S citizenship or alien status, and their authorization to work in the United States.*

ASSISTANT PROFESSOR, biology (tenure-track) and **ASSISTANT PROFESSOR** (tenure-track), or **ASSOCIATE PROFESSOR** (tenured), environmental science. Interdisciplinary arts and sciences, University of Washington, Bothell. Seeking candidates for two full-time appointments to teach natural science in an interdisciplinary program, with preference given to those conducting research with applications to social issues and bioethics (biology) or to enhance environmental, social, and cultural sustainability (environmental science). Ph.D. in biology or Ph.D. in environmental science (or appropriate natural science field) required and two years teaching experience preferred. Applications should include a research and teaching statement of qualifications for working in an interdisciplinary program, a representative publication, curriculum vitae, three letters of recommendation, and a sample syllabus from an interdisciplinary course. For more information, see **website: http://www.uwb.edu.** Address applications by 1 November 2005 to: **Pam DePriest, Science Searches, Box 358530, University of Washington/Bothell, 18115 Campus Way N.E., Bothell, WA 98011.** *The University of Washington is an Affirmative Action/Equal Opportunity Employer.*

PROFESSOR AND HEAD, PLANT PATHOLOGY
Kansas State University

Kansas State University invites nominations and applications for the position of Head, Department of Plant Pathology. The Head administers personnel, physical facilities, and budgets for teaching, research, and extension programs. Engagement in an active teaching, research, or extension program is possible. Applicants must have a Ph.D. in a biological science area relevant to plant pathology, effective communication skills, and national recognition in instruction, research, or extension. Please submit a letter of application, including a statement of leadership philosophy, curriculum vitae, and the names, addresses, and telephone numbers of five references to: **Sonny Ramaswamy, Department of Entomology, Kansas State University, Manhattan, KS 66506. E-mail: sonny@ksu.edu.** Review of applications will begin October 1, 2005, and continue until the position is filled. For more information concerning the position and the Department, see **website: http://www.oznet.k-state.edu/plantpath/.** *Kansas State University is an Equal Opportunity/Affirmative Action Employer.*

CELL BIOLOGIST
TENURE-TRACK ASSISTANT PROFESSOR

The Department of Biology (**website: http://www.uwlax.edu/biology/**) at the University of Wisconsin (UW)-La Crosse invites applications for an academic year, tenure-track position at the level of Assistant Professor. Candidates must have a strong commitment to undergraduate education. Teaching responsibilities will include radiation biology, biology of cancer, a course in the candidate's area of expertise, and participation in teaching one or more of the following: cell biology laboratory, genetics laboratory, introductory biology. Ph.D. in a biological science is required. Some previous teaching experience is desirable. The successful candidate will be expected to develop an externally funded research program (cancer biology preferred) and direct undergraduate and graduate (M.S.) research. Academic year salary competitive and commensurate with experience. Start August 29, 2006. Applicants should submit letter of application, curriculum vitae, statements of teaching philosophy and research interests, graduate and undergraduate transcripts, and three letters of recommendation to: **Dr. Mark Sandheinrich, Department of Biology, University of Wisconsin-La Crosse, La Crosse, WI 54601.** Applications must be received by October 15, 2005. If you have a special need/accommodation to aid your participation in our hiring process, please contact Mark Sandheinrich to make appropriate arrangements. *UW-La Crosse is an Affirmative Action/Equal Opportunity Employer. Women, persons of color, and individuals with a disability are encouraged to apply.*

ORGANIC CHEMISTRY
Dartmouth College

Applications are invited for a faculty position at the **ASSISTANT PROFESSOR** level starting July 2006. The Chemistry Department seeks an individual who will establish a nationally recognized research program in organic chemistry at Dartmouth, and who will excel at teaching in our undergraduate and Ph.D. curriculum. Candidates will be expected to be able to teach introductory and advanced courses in organic chemistry, as well as graduate courses in their area of research. Applicants should submit curriculum vitae, a description of their research plans, and a brief statement about their teaching interests. Applicants should also arrange to have three letters of recommendation sent on their behalf. All inquiries and applications will be treated confidentially. Application materials should be sent to: **Chair, Organic Chemist Search Committee, Department of Chemistry, 6128 Burke Laboratory, Dartmouth College, Hanover, NH 03755-3564.** The Committee will begin to consider completed applications on October 15, 2005. *With an even distribution of male and female students and over a quarter of the undergraduate student population members of minority groups, Dartmouth is committed to diversity and encourages applications from women and minorities. Dartmouth College is an Equal Opportunity/Affirmative Action Employer.*

ASSISTANT PROFESSOR
Marine Invertebrate Physiology

McDaniel College invites applications for a tenure-track appointment at the level of Assistant Professor in marine invertebrate physiology to begin fall 2006. Responsibilities include courses in animal physiology, invertebrate zoology and marine biology, senior research projects. Ph.D. required. Interested applicants should send a letter of application, curriculum vitae, three letters of reference, statement of teaching philosophy, and research interests, including areas for student-faculty research to: **Dr. Louise Paquin, Biology Department, McDaniel College, 2 College Hill, Westminster, MD 21157-4390.** Review of applications will begin September 26, 2005. See **website: http://www.mcdaniel.edu/hr/facultyjoblistings.shtml.** *McDaniel College, an Affirmative Action/Equal Employment Opportunity and award-winning ADA Employer, welcomes applications from women and men of diverse racial/ethnic backgrounds.*

POSITIONS OPEN

GENETICS TENURE-TRACK POSITION

Truman State University invites applications for a tenure-track **ASSISTANT PROFESSOR** faculty position in genetics, starting August 2006. The successful candidate will teach a sophomore-level genetics course, introductory biology and, depending on specialty, an upper-level elective course(s). Research area in genetics is open; candidates with expertise in developmental or bacterial genetics are encouraged to apply.

Candidates should be strongly committed to the 'teacher-scholar' model in a liberal arts and sciences institution and to maintaining both quality teaching and an active research program. A research laboratory in our new Science building and competitive startup funds will be provided. To review a more detailed position announcement, please visit **website: http://www.truman.edu/pages/152.asp.** For more information about the University and the Biology program, please visit **websites: http://www.truman.edu** and **http://biology.truman.edu.**

Candidates should possess a Ph.D. by August 2006. Complete applications include: letter of application; current curriculum vitae; statement of teaching philosophy and commitment to the liberal arts and sciences and student development; statement of research interests and goals; three recent letters of recommendation; and all graduate and undergraduate transcripts (copies acceptable, official copies of graduate transcripts required prior to hiring). All application materials should be sent to: **Dr. Jeffrey Osborn, Genetics Search, Division of Science, Truman State University, 100 E. Normal Street, Kirksville, MO 63501-4221. Telephone: 660-785-4017.** Review of complete applications will begin September 20, 2005.

Truman is an Equal Employment Opportunity/Affirmative Action/ADA Employer.

RESEARCH PROGRAMMER/SCIENTIST

Penn State's Information Technology Services' Graduate Education and Research Services group is seeking outstanding computational scientists to provide high-performance computing consultation to faculty for research and instruction. In collaboration with faculty in several colleges, the selected candidates will be able to initiate and pursue research projects, submit grant applications, teach seminars and courses, and publish papers. Applicants must have academic training and computing expertise in one or more of the following areas: mathematics and statistics, computer science, business logistics, database administration and application, bioinformatics, engineering, and scientific modeling and applications, computational chemistry and materials science, grid computing, middleware, visualization, parallel programming. Excellence of a candidate's computational experience is more important than their emphasis on any particular discipline. Minimum qualifications: Bachelor's degree plus four years of related experience or an equivalent combination of education and experience; Master's degree with experience or a Ph.D. degree in a related academic discipline is strongly preferred. Knowledge of programming languages, experience with operating systems and various software tools, excellent written and interpersonal communications skills, and self-motivation are essential. For a more detailed description of this position and application procedure, please visit **website: http://gears.aset.psu.edu/employment.** *Penn State is committed to Affirmative Action/Equal Opportunity, and the diversity of its workforce.*

Biology. **ASSISTANT PROFESSOR.** August 2006. Genetics and molecular biology (also physiology; general biology). Ph.D. (or All But Dissertation) required. Focus on undergraduate teaching and research. Review commences October 1, 2005. Send curriculum vitae, teaching philosophy, research interests, and reference letters to: **Paul H. Blaney, Dean of Faculty, Emory and Henry College, P.O. Box 947, Emory, VA 24327** or **e-mail: pblaney@ehc.edu.** *Affirmative Action/Equal Employment Opportunity.*

POSITIONS OPEN

AQUATIC BIOLOGIST, ASSISTANT PROFESSOR. The Biology Department of Hobart and William Smith Colleges invites applications for a tenure-track position for the 2006-2007 academic year. Candidates must have a Ph.D. degree by the starting date. Individuals with broad experience in freshwater biology and ecosystems ecology are encouraged to apply. Responsibilities include teaching a course in aquatic ecology, a course in the applicant's specialty, participating in an introductory biology course, and contributing to the Colleges' general curriculum. The candidate is expected to demonstrate excellence in teaching and implement a research program that involves undergraduates and makes use of local freshwater ecosystems. The candidate will also contribute to the Colleges' Environmental Studies Program. Experience and/or desire for working in a multicultural environment are desirable Hobart and William Smith Colleges are committed to attracting and supporting a faculty of women and men that fully represent the racial, ethnic, and cultural diversity of the nation, and actively seek applications from underrepresented groups. Hobart College for men and William Smith College for women are coordinate residential colleges that share a campus on Seneca Lake in the Finger Lakes region of New York. The Colleges own a 65-foot research vessel on Seneca Lake and are home to the Finger Lakes Institute, a regional center for environmental research and education. Applicants should send curriculum vitae, a statement of teaching philosophy, a statement of research interests, official transcripts for all degrees received, and three letters of reference to: **Dr. David Droney, Biology Department, Hobart and William Smith Colleges, Geneva, NY 14456.** To ensure full consideration, applications should be received by 7 October 2005.

For further information on the Colleges' Biology Department, see our **website: http://www.hws.edu/aca/depts/bio/index.html.**

TENURE-TRACK POSITION
BIOCHEMISTRY
University of Wisconsin-Madison

The Department of Biochemistry at the University of Wisconsin-Madison invites applications for a position in biochemistry at the **ASSISTANT PROFESSOR** level. Applications in all areas of biochemistry will be considered. The University and Department provide an excellent environment for the development of an outstanding research program. The successful candidate will be expected to develop a vigorous, extramurally funded, independent research program, and to participate in the undergraduate and graduate teaching programs of the Department. They will also be expected to perform University and community service as appropriate. Applications, including curriculum vitae, list of publications, and a brief summary of accomplishments and direction of future research and three letters of reference should be sent to: **Professor Elizabeth Craig, Chair, Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706-1544.** Applications should be received by October 15, 2005, to ensure consideration. *The University of Wisconsin is an Affirmative Action/Equal Opportunity Employer and encourages applications from women and minorities.*

MOLECULAR NEUROBIOLOGIST

With expertise in regeneration research and stem cell technology. A background in developmental neurobiology is a plus. Research to be conducted in collaboration with clinical neuroscientists. Mentoring residents in neurological surgery in research techniques is expected. Competitive salary and benefits. Please submit curriculum vitae and letters of interest to: **Setti Rengachary, M.D., Department of Neurological Surgery, Wayne State University, 4160 John R, Suite 930, Detroit, MI 48201. E-mail: srengachary@med.wayne.edu.**

POSITIONS OPEN

SOIL ECOLOGIST

The Department of Environmental and Plant Biology at Ohio University invites applicants at the **ASSISTANT PROFESSOR** level for a full-time, tenure–track appointment beginning September 2006. Candidates must be committed to both undergraduate and graduate education. Applicants with experience in any area of below–ground dynamics are encouraged to apply; however, interest in root dynamics, plant–soil interactions, soil food webs, fungi, and/or spatial statistics would be particularly advantageous and complement the existing research group. Willingness to participate in the existing Forest Ecology Research Group, which emphasizes temperate deciduous forest ecosystems, is essential. Applicants should have postdoctoral experience and a demonstrated ability to develop a strong, externally funded research program. Teaching responsibilities will likely include an introductory nonmajors biology course, an upper-level soil biology course, and an upper-level course in restoration ecology. Submit cover letter, curriculum vitae, statements of teaching philosophy and research interest, and three letters of recommendation to: **Chair of the Search Committee, Department of Environmental and Plant Biology, Ohio University, Porter Hall 315, Athens, OH 45701-2979.** Review of applications begins September 30, 2005, and continues until the position is filled. Direct inquiries to: **Gar W. Rothwell, Chair (e-mail: rothwell@ohio.edu or fax: 740-593-1130).** Information about the Department, College, and University available at **websites: http://www.plantbio.ohiou.edu** and **http://www.ohio.edu.** *The University places a high priority on the creation of an environment supportive of the promotion of women, minorities, veterans, and persons with disabilities.*

Ohio University is an Equal Opportunity/Affirmative Action Employer.

ASSISTANT PROFESSOR, CHEMISTRY

The Department of Chemistry of the University of Wisconsin-Madison anticipates an opening for a faculty position to begin in August 2006. We seek outstanding candidates at the Assistant Professor level (tenure track) in all areas of chemistry including chemical education. Candidates must have a Ph.D. in chemistry or a related field; postdoctoral experience is desirable. The position requires development of an internationally recognized program of scholarly research as well as excellent teaching at both the undergraduate and graduate levels. Please submit curriculum vitae and concise description of research plans online at **website: http://www.chem.wisc.edu.** Three letters of recommendation will also be required through the online service directed to: **Chair, Faculty Search Committee, Department of Chemistry, University of Wisconsin-Madison, 1101 University Avenue, Madison WI 53706-1322.** To guarantee full consideration, all materials must arrive before October 1, 2005.

The University of Wisconsin is an Equal Opportunity/Affirmative Action Employer; applications from qualified women and minority candidates are encouraged. Unless confidentiality is requested in writing, information regarding the identity of the applicant must be released on request. Finalists cannot be guaranteed confidentiality.

The Department of Chemistry at The University of Chicago invites applications from outstanding individuals for the position of **ASSISTANT PROFESSOR** of chemistry. This search is in the areas broadly defined as inorganic, organic, and physical chemistry. Applicants must mail hard copies of curriculum vitae, a list of publications, and a succinct outline of their research plans; and arrange for three letters of recommendation to be sent by mail to: **Michael D. Hopkins, Chairman, Department of Chemistry, The University of Chicago, 5735 S. Ellis Avenue, Chicago, IL 60637.** Review of completed applications will begin October 1, 2005; to ensure full consideration, all materials should be submitted by that date. *An Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

COMMUNITY OR ECOSYSTEM ECOLOGIST

The Department of Biological Sciences at Texas Tech University invites applications for a tenure-track ecology position at the **ASSISTANT PROFESSOR** level beginning September 2006. We seek a broadly trained ecologist without regards to taxon or system with theoretical or empirical research interests that may include quantitative ecology, ecological modeling, and theoretical ecology at the level of the community or ecosystem. Candidates are expected to demonstrate the motivation and ability to develop a vigorous, competitive research program, participate in multidisciplinary research that may integrate with current faculty in ecology, train and mentor undergraduate and graduate students, and contribute to graduate and undergraduate teaching. Teaching responsibilities will include introductory ecology, a general course in biology, and upper-level and graduate courses in the candidate's research area. Ph.D. required; postdoctoral experience preferred. Applicants should submit a cover letter, curriculum vitae, and descriptions of research goals and teaching interests and philosophy, and should arrange for three letters of recommendation to be forwarded. Applications can be submitted online at **website: http://jobs.texastech.edu,** by **e-mail: lanita.ladd@ttu.edu,** or mailed to: **Ecologist Search, c/o John Zak, Chair, Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131.** For further information, contact: **Dr. David Tissue, Chair, Ecology Search Committee** at **e-mail: david.tissue@ttu.edu.** Applications should be received by October 14, 2005. Visit our **website: http://www.biol.ttu.edu.** *Women and members of underrepresented groups are encouraged to apply.*

DEVELOPMENTAL BIOLOGIST ASSISTANT PROFESSOR
University of Denver

The Department of Biological Sciences, University of Denver, invites applications for a tenure-track position at the Assistant Professor level to begin September 1, 2006. Candidates using vertebrate or invertebrate model systems relevant to developmental biology are sought. The successful candidate will have a Ph.D. and postdoctoral experience in appropriate fields, will develop an extramurally funded research program, will supervise undergraduate research projects and M.S. and Ph.D. students, and will teach undergraduate and graduate courses in areas of specialty. Submit curriculum vitae, two recent publications, three letters of recommendations, and statements of (a) teaching philosophy and (b) research interests to: **Dr. Susan Sadler, Chair, Developmental Biologist Search Committee, Department of Biological Sciences, University of Denver, Denver, CO 80208.** Applications should be received by October 15, 2005. Information about the Department of Biological Sciences can be found at **website: http://www.biology.du.edu.** *The University of Denver is an Equal Opportunity/Affirmative Action Employer.*

RESEARCH SCIENTIST

The Department of Pharmacology, School of Medicine at Stony Brook University, located on the Northshore of Long Island, is seeking a Research Scientist for a study of atherosclerosis and Apo E signaling. Required: Ph.D., D.Sc. in a biological science or related field or M.D. and four years of postdoctoral experience. Mechanisms of atheroprotection in KO/transgenic mice will be explored at the cellular level. Experience in cell signaling (including RNA expression analysis/proteomics) preferable. To apply, please send curriculum vitae, three letters of reference, and cover letter to: **Drs. Fayanne Thorngate and Craig C. Malbon, Department of Pharmacology, Stony Brook University, Stony Brook, NY 11794-8661. E-mail: craig@pharm.stonybrook.edu.** Visit **website: http://www.stonybrook.edu/cjo** for employment information. *Affirmative Action/Equal Opportunity Employer.*

FACULTY POSITION, BIOANALYTICAL CHEMISTRY
Department of Chemistry
The University of Kansas

The University of Kansas (KU) is expanding its commitment to research in the life sciences. The Chemistry Department invites applications for a tenure-track faculty position beginning August 18, 2006, or thereafter. The position is at the **ASSISTANT PROFESSOR** level, although exceptional candidates at the **ASSOCIATE PROFESSOR** level may also be considered. A Ph.D. in analytical chemistry or a closely related field is required and postdoctoral experience is desirable. Duties include teaching at the undergraduate and graduate levels and the direction of a vigorous research program in bioanalytical chemistry. Assistant level candidates need to provide a well-defined research plan, evidence of teaching ability, and have a desire to work in and contribute to the exciting multidisciplinary life sciences research environment at KU. Associate level candidates must show evidence of an active research program. Salary will be commensurate with qualifications and experience. Applicants should submit a letter of interest, curriculum vitae, a brief summary of two to three research proposals of one to three pages each (senior candidates should provide a brief description of research goals for the next five years and a description of current personnel and funding resources, including research grants), and should arrange for the submission of at least three letters of recommendation to: Professor Craig Lunte, Analytical Search Committee Chair at **e-mail: clunte@ku.edu** or **telephone: 785-864-4313.** Initial review of applications will begin November 1, 2005, and will continue until the position is filled. Paid for by KU.

University of Kansas
Department of Chemistry
Room 2010
1251 Wescoe Hall Drive, Malott Hall
Lawrence, KS 66045

Equal Opportunity/Affirmative Action Employer.

JUNIOR FACULTY POSITION, PROSTATE CANCER METASTASIS RESEARCH
University of Michigan Medical School

We are seeking a colleague to join us in a tenure-track position as an **ASSISTANT PROFESSOR** of medicine in the Division of Hematology/Oncology. The successful applicant will join a core group of research investigators from multiple disciplines studying mechanisms underlying prostate cancer metastasis/bone microenvironment interactions. We are seeking an investigator with expertise in the area of signal transduction and prostate cancer metastasis. Applicants are expected to have a Ph.D. (or equivalent) and at least two years of postdoctoral experience in the field of prostate cancer metastasis. Applicants should have a demonstrated ability to communicate and have a record of publications in the field. A history of funding at the postdoctoral level is preferred. To be considered, please submit curriculum vitae, publication list, and a statement of research accomplishments. Completed applications must be received by September 1, 2005. Applications should be submitted to: **Kenneth J. Pienta, M.D., 7308 CCGC, 1500 E. Medical Center Drive, Ann Arbor, MI 48109.** *The University of Michigan is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL FELLOW IN DIABETES RESEARCH

Fellowship available to study diabetic cardiomyopathy and diabetic nephropathy in transgenic mouse models. Projects are funded by multiple NIH grants to understand mechanisms of diabetes induced cell and organ damage and changes in gene expression. Applicants must have a Ph.D. and strong record of publications. Send curriculum vitae, summary of research interests, and the names of three references to:

Dr. Paul N. Epstein
University of Louisville
E-mail: paul.epstein@louisville.edu

UNIVERSITY OF NEBRASKA MEDICAL CENTER (UNMC)-OMAHA
Redox Biology of Neurodegenerative and Neuropsychiatric Disorders

The Redox Biology and Neurovirology and Neurodegenerative Disorders Centers (RBC and CNND) have created an exciting opportunity for an exceptional Ph.D., M.D., or M.D./Ph.D. with joint research interests in redox biology and neurodegenerative or neuropsychiatric disorders. Cutting-edge neuroimaging, proteomics, genomics, electrophysiology, flow cytometry, and neural cell core facilities complement an excellent startup package. A state-of-the-art biomedical research center offers spacious modular laboratories, on-site animal facilities, and administrative support. The successful candidate will have a significant academic record and a willingness to perform interdisciplinary research. The position is "open rank" and complements established NIH-funded laboratories in neuroimmunology, redox biology, HIV-associated dementia, amyotrophic lateral sclerosis, depression, and Alzheimer's and Parkinson's Disease. Faculty appointments are to the Department of Pharmacology and Experimental Neuroscience (primary) and the RBC and CNND (secondary). For full consideration, interested persons should send curriculum vitae, a succinct description of research plans, and names of three references by October 1, 2005, to: **Anuja Ghorpade, Department of Pharmacology and Experimental Neuroscience, Department 985800, Nebraska Medical Center, Omaha, NE 68198. Fax: 402-559-7495; e-mail: neuro-redox@unmc.edu.**

Affirmative Action/Equal Opportunity Employer.

WELLESLEY COLLEGE
Wellesley, Massachusetts

The Program in Neuroscience at Wellesley College invites applications for a tenure-track faculty position at the rank of first-level **ASSISTANT PROFESSOR** beginning in September 2006. We are seeking candidates who are committed to excellence in both teaching and research in a liberal arts environment. Candidates will be expected to teach courses at all levels of our curriculum and should have plans for an active research agenda that involves undergraduates. While the position is open to any field of neuroscience, we are especially interested in candidates whose work includes a neuropharmacology component. A Ph.D. and postdoctoral experience are required. Applications should include curriculum vitae, statements of teaching and research interests, and three letters of recommendation. Review of applications will begin November 1, 2005. Candidates who believe they will contribute to that goal are encouraged to apply. Submit applications to: **Search Committee, Neuroscience Program, Wellesley College, 106 Central Street, Wellesley, MA 02481.** *Wellesley College is an Equal Opportunity/Affirmative Action Educational Institution and Employer. The College is committed to increasing the diversity of the College community and the curriculum.*

Loma Linda Veterans Association for Research and Education seeks **RESEARCH ASSOCIATES** in our Loma Linda, California, location. Molecular: Do studies on regulation of osteoclast resorption and other bone biology studies. Working in molecular, cell, and/or bone biology, including current DNA sequencing procedures, RNA analysis, immunocytochemistry and cloning, viral vectors, and nucleic acid and protein database. Must have Ph.D. in molecular biology or related field. Microbiological: Perform basic research in fields of molecular biology and genetics, focus is on genetic loci and gene isolation. Must have Ph.D. in microbiology or related field plus one year relevant experience.

All researchers will design and conduct experiments in consultation with senior investigators, interpret and present results of these experiments, prepare manuscripts based on results. Train technical personnel. Resume to: **Human Resources Manager, P.O. Box 1280, Redlands, CA 92373.**

ZOOLOGY/BIOMETRY
Bridgewater State College
Bridgewater, MA

Position: Assistant Professor, Tenure Track, Zoology/Biometry in the Department of Biological Sciences.

Responsibilities: The position requires teaching introductory zoology for both majors and non-majors, a core course in biometry, and upper level courses to augment the department's offerings. In addition, the occupant of this position will serve as a consultant in biometry to the departmental undergraduate research program. Advising students and supervising original undergraduate research are also required.

Qualifications: The successful candidate must have an earned Ph.D. by May 2006 and excellent communication skills. Training and experience in both organismal zoology and biometry are required, with background in both vertebrate and invertebrate zoology. A strong commitment to teaching, research, and advising in an undergraduate setting is also required. Teaching experience is preferred as are post-doctoral research and grant experience.

TO APPLY: Please visit our career site and apply online: **https://jobs.bridgew.edu**. Review of applications will continue until the position is filled. For more information about employment at Bridgewater State College, please visit our website at: **http://www.bridgew.edu/HR/JobList/**.

USDA ARS Agricultural Research Service
www.ars.usda.gov

RESEARCH MOLECULAR BIOLOGIST, GS-12/13
Salary Range: $62,886 - $97,213 per annum PLUS benefits package

The Foreign Disease-Weed Science Research Unit, Fort Detrick, Maryland, is seeking a permanent, full-time scientist to perform research in molecular genetics, characterization, and detection of emerging fungal pathogens of crops and ornamental plants. Primary responsibility will be to characterize isolates of Asian soybean rust. Work will be performed in a BSL-3 Level Biological Containment Facility which requires a preemployment security check and a full background investigation. Appropriate education and/or experience in molecular biology are required. U.S. citizenship is required.

Candidates must request a copy of vacancy announcement **ARS-X5E-0281** by either calling **301-504-1482** or via the internet **http://www.afm.ars.usda.gov/hrd/jobs/index.htm** in order to address specific information outlined in the vacancy announcement. Applications must be postmarked by **October 17, 2005**.

USDA/ARS is an Equal Opportunity Provider and Employer.

UNIVERSITY OF MARYLAND

THE DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

Bio-molecular X-ray Crystallographer

All Levels Tenure-track or tenured position (Assistant Professor, Associate Professor, and Professor)

As part of major University initiatives in the life sciences and biophysics, the Department of Chemistry and Biochemistry seeks to appoint a bio-molecular x-ray crystallographer to a tenured or tenure-track position. We seek outstanding scientists whose research interests complement existing strengths in the department and across the University and who are committed to developing outstanding academic programs in research and teaching. One of four departments within the College of Chemical and Life Sciences, members of the Department of Chemistry and Biochemistry participate in university centers and initiatives that include the Center for Biomolecular Structure and Organization, the Center for Bioinformatics and Computational Biology, the Institute for Physical Science and Technology, as well as a university-wide initiative in biophysics. The University of Maryland, College Park is the flagship campus of the University of Maryland System and is ideally situated in close proximity to Washington, D. C., Baltimore, and Maryland's 270 Technology Corridor. Government labs including NIH, NRL, FDA and NIST are also nearby. Candidates should submit a curriculum vitae, a three-page summary of research plans, a statement of educational interests, and contact information for three persons from whom letters of recommendation can be requested. Submit applications via the department web site (**http://www.chem.umd.edu/employment.html**).

Qualifications: We seek scholars who will build or have highly visible, widely acclaimed research programs and who are capable of excellence in undergraduate and graduate education. Candidates are expected to have a Ph.D. degree, demonstrated accomplishments in independent research, and promise as an effective educator in the chemical sciences.

Salary: Commensurate with qualifications.

Deadline: Review of applications will begin **October 11, 2005**, but we will continue to accept applications until the position is filled.

AN EQUAL OPPORTUNITY, AFFIRMATIVE ACTION EMPLOYER. APPLICATIONS FROM WOMEN AND MINORITIES ARE ENCOURAGED.

UNIVERSITY OF CALIFORNIA SANTA BARBARA FACULTY POSITION IN CHEMISTRY AND BIOCHEMISTRY

The Department of Chemistry and Biochemistry at the University of California Santa Barbara invites applications for a tenure-track faculty position to begin July 1, 2006 at the level of Assistant Professor. Outstanding candidates with research and teaching interests in all sub-areas of organic chemistry including bio-organic, physical-organic, chemical biology and other areas intersecting with organic chemistry are invited to apply. Applicants may consider building campus-wide interactions and alliances with interdisciplinary programs, University-based Centers and Institutes, and other departments within the Colleges of Letters and Sciences and Engineering.

Applicants should submit their curriculum vitae, a description of their research plans, graduate and undergraduate teaching interests, and arrange to have three letters of recommendation sent on their behalf to **The Organic Faculty Search Committee, Department of Chemistry and Biochemistry, University of California Santa Barbara, Santa Barbara, CA 93106-9510**. Review of applications will begin **October 15, 2005** and will continue until the position is filled. A Ph.D. is required at the time of appointment.

The department is especially interested in recruiting candidates who can contribute to the diversity and excellence of the academic community through research, teaching and service. The University of California is An Equal Opportunity/Affirmative Action Employer.

UNIVERSITY OF CALIFORNIA SANTA BARBARA FACULTY POSITIONS IN CHEMISTRY AND BIOCHEMISTRY

The Department of Chemistry and Biochemistry at the University of California Santa Barbara announces a search for a tenure-track faculty member to begin in Fall 2006 at the Assistant Professor level. Outstanding candidates with research and teaching interests in all sub-areas of inorganic chemistry including bioinorganic, inorganic materials and organometallic chemistry are invited to apply. In addition to contributing to the research, teaching and service missions of the Department of Chemistry and Biochemistry, we anticipate campus-wide interactions in interdisciplinary programs between departments in the Colleges of Letters and Sciences and Engineering, along with other University-based Centers and Institutes.

Applicants should submit a curriculum vitae, description of their research plans, graduate and undergraduate teaching interests, and arrange to have three letters of recommendation sent on their behalf to **Faculty Search Committee, Department of Chemistry and Biochemistry, University of California Santa Barbara, Santa Barbara, CA 93106-9510**. Review of applications will begin **October 1, 2005** and will continue until the position is filled. A Ph.D. is required at the time of appointment.

The department is especially interested in candidates who can contribute to the diversity and excellence of the academic community through research, teaching and service. The University of California is An Equal Opportunity/ Affirmative Action Employer.

POSITIONS OPEN

USDA ARS Agricultural Research Service
www.ars.usda.gov

The Agricultural Research Service, Plant Sciences Institute, Systematic Entomology Laboratory, U.S. National Museum of Natural History in Washington, D.C., is seeking two **RESEARCH ENTOMOLOGISTS** to study and characterize parasitic Hymenoptera by analyzing molecular, morphological, biological, and behavioral characters. Salary is commensurate with experience (salary range GS-12: $62,886 to $81,747 and GS-13: $74,782 to $97,213 per annum) plus benefits. *U.S. citizenship is required.* Positions require education in entomology, plus (1) knowledge of theories, principles, and methodology for the study of Hymenoptera; (2) knowledge of rules of nomenclature, cladistics, and other principles of systematic biology; (3) skill in analyzing molecular and morphological characters of Hymenoptera; and (4) ability to design, plan, and conduct research, and publish results in peer-reviewed journals. For research information, contact: **Dr. Alma Solis** at **telephone: 301-504-5183.** Candidates must request a copy of vacancy announcement ARS-X5E-0307 by either calling **telephone: 301-504-1482** or via **website: http://www.afm.ars.usda.gov/hrd/jobs/apply.htm.**

ASSISTANT PROFESSOR GENOMICS: QUANTITATIVE, POPULATION, OR COMPARATIVE

The Biology Department at University of Kentucky seeks a tenure-track Assistant Professor with expertise in genomics. Candidates that integrate experimental and computational approaches to study populations, complex traits, or genomes are especially encouraged to apply. The Department will consider applications from a wide range of specializations including, but not limited to, bioinformatics, development, environmental biology, evolution, genetics, and neurobiology. Applicants must provide evidence that they will develop an active, independently funded research program. A commitment to teaching and student training is expected. Applicants should submit curriculum vitae and a statement detailing their current and future research plans, and arrange for submission of three letters of recommendation. Please address applications to:

Randal Voss, Chair
Genomics Search Committee
Department of Biology
University of Kentucky
101 TH Morgan Building
Lexington, KY 40506

Applications must be received by October 15, 2005, to ensure full consideration.

Equal Opportunity/Affirmative Action Employer. Women and minorities encouraged to apply.

ASSISTANT PROFESSOR Microbiology and Cell Science University of Florida

The Department of Microbiology and Cell Science at the University of Florida invites applications for an Assistant Professor tenure-track position to develop an externally funded research program in microbiology. Candidates with interests in evolutionary microbiology, systems biology, or structure-function relationships of microbial model systems or host-microbe interactions are especially encouraged to apply. Applicants must have a Ph.D., postdoctoral experience, and a strong publication record. The successful candidate is expected to develop an outstanding program and participate in our undergraduate and graduate programs. Details of the Department and the position may be found at **website: http://microcell.ufl.edu.** Submit applications as a single PDF file containing a cover letter, curriculum vitae, and summary of research interests to: **Dr. Madeline Rasche (e-mail: mrasche@ufl.edu).** Three letters of reference should also be sent directly to **e-mail: mrasche@ufl.edu.** Review of applications will begin October 15, 2005. *The University of Florida is an Equal Opportunity Employer.*

POSITIONS OPEN

The Department of Chemistry at The University of Toledo invites applications or nominations for three tenure-track **FACULTY POSITIONS** in (1) bioanalytical chemistry, (2) surface characterization of thin films, and (3) macromolecular crystallography beginning August 2006. These hires are part of a university-wide strategic hiring plan to enhance existing research strengths in biological and materials chemistry across the University. A Ph.D. degree in chemistry or a related field is required; postdoctoral experience is preferred. The successful candidates will be expected to develop a vigorous, externally funded research program, to have a commitment to excellence in teaching at both the undergraduate and graduate (M.S. and Ph.D.) level and to participate in University-wide research initiatives. The University is a comprehensive state institution with an enrollment of approximately 20,000 students located on an attractive campus in suburban Toledo. The University offers competitive salaries and an excellent benefits package for its faculty. Further information about the University, the Department, and the Instrumentation Center at The University of Toledo is available through the departmental website (**website: http://www.chem.utoledo.edu**). Applicants should send their curriculum vitae, a research plan, and arrange for three letters of recommendation to be sent to the appropriate search committee:

Chair, Department of Chemistry
University of Toledo
2801 W. Bancroft Street
Toledo, OH 43606

Review of applicants will begin on October 15, 2005, and continue until the position is filled. *The Department encourages applications from minorities, women, and persons with disabilities. The University of Toledo is an Affirmative Action/Equal Opportunity Employer, Minorities/Females/Persons with Disabilities/Veterans.*

SUPERVISORY BIOLOGIST

The Pacific Island Ecosystems Research Center is seeking applications for a permanent, full-time position as Center Director. This is a unit of the Biological Resources Discipline of the U.S. Geological Survey (USGS) located in Honolulu, Hawaii (Oahu) or Hawaii National Park, Hawaii (Big Island). The primary duties will include: scientific research on marine and terrestrial biology including watershed ecology, invasive species, biological control, avian and plant pathogens and disease, and endangered species restoration throughout the Pacific Basin. Salary ranges from $89,625 to $116,517 per year plus benefits plus COLA of 25% (Oahu) or 16.5% (Big Island).

To apply go to the U.S.A. jobs **website: http://www.usajobs.opm.gov** and locate the following announcements (open August 19, 2005, through September 30, 2005): WR-2005-0488 Supervisory Biologist GS-0401-15; WR-2005-0489 Supervisory Biologist GS-0401-15.

The USGS is an Equal Opportunity Employer. Selection for this position will be based solely on merit, fitness, and qualifications without regard to race, sex, color, religion, age, marital status, national origin, nondisqualifying handicap conditions, sexual orientation, or any other nonmerit factors. This agency provides reasonable accommodation to applicants with disabilities.

HEAD of manufacturing and process development. Responsible for all vaccine manufacturing, process, and product development. Direct, perform, and oversee construction of Bacillus Camille Guerin (BCG) manufacturing facility and other laboratory related facilities. Direct and oversee process development and scale-up activities for large-scale BCG by fermentation. Requirements: M.S. in biological sciences (or foreign equivalent) and experience in biological manufacturing including large scale fermentation and production of BCG using stirred tanks. Job in Rockville, Maryland, location. Send resume to: **Joanna Lathrop, Human Resources (e-mail: hr@aeras.org or fax: 301-547-2903), Aeras Global TB Vaccine Foundation, 7500 Old Georgetown Road, Suite 800, Bethesda, MD 20814.**

POSITIONS OPEN

USDA ARS Agricultural Research Service
www.ars.usda.gov

RESEARCH GENETICIST (INSECTS) GS-12/13/14

Salary range of $60,576 to $110,662 per year

The Fruit and Vegetable Insect Research Unit, Wapato, Washington, is seeking a permanent full-time Research Geneticist (insects) to work in the area of developing, testing, and applying transgenic and/or other molecular techniques to create new, and/or enhance existing, biological-based control strategies for major tree fruit pests. See **website: http://www.ars.usda.gov/pwa/yarl.** We are located near Yakima, Washington. A Ph.D. or equivalent in genetics or a related field is highly desirable. This position qualifies for health benefits, life insurance, annual and sick leave, and Thrift Savings benefits. Vacancy announcement number: ARS-X5W-0391. For details and application instructions, see **website: http://www.afm.ars.usda.gov/divisions/hrd/index.html.** Announcement closes October 7, 2005. To receive a printed copy, call **telephone: 509-454-6575.** *U.S. citizenship is required.* Visit the ARS **website: http://www.ars.usda.gov.** *USDA/ARS is an Equal Opportunity Employer and Provider.*

DIRECTOR, WATER QUALITY RESEARCH LABORATORY

Heidelberg College, Tiffin, Ohio, invites applications for the position of Director of its nationally recognized Water Quality Laboratory (WQL). The WQL's research programs focus on quantifying nutrient, sediment, and pesticide export from large agricultural and mixed land-use watersheds into Lake Erie and the Ohio River, aiding in the development of tributary load reduction programs, and assessing the effectiveness of those programs. The WQL's tributary loading data bases stretch back to 1974 and are the most detailed and long-term of their type in the United States. In January 2005, the WQL moved into the newly constructed Gillmor Science Hall on the Heidelberg campus. We seek a Director who can guide the continued operation and expansion of WQL programs. Applicants must possess a doctoral degree with experience in water resources or a related environmental or agricultural discipline. More information about this position and WQL programs and staff can be found at **website: http://www.heidelberg.edu/wql.** The Director holds a non-teaching position and reports directly to the Vice President for Academic Affairs. To apply, submit by mail (1) a letter of application, (2) a full curriculum vitae, (3) a one- to two-page narrative envisioning how you would lead the WQL in implementing its mission, and (4) names and contact information for three references. Submit these materials to:

Dr. David Baker
Water Quality Laboratory
Heidelberg College
310 E. Market Street
Tiffin, OH 44883

Screening of applications will begin October 1, 2005, and will continue until the position is filled. *Heidelberg College is an Affirmative Action/Equal Opportunity Employer.*

The Department of Chemistry at the University of Chicago invites applications from qualified individuals for positions of **POSTDOCTORAL RESEARCH ASSOCIATE** in chemistry. These searches are in the areas broadly defined as inorganic, organic, and physical chemistry. For details about specific job opportunities and how to apply visit **website: http://jobopportunities.uchicago.edu.** Qualified applicants will have a Ph.D. degree or will have completed the Ph.D. requirements in the related areas prior to hire. *The University of Chicago is an Equal Opportunity/Affirmative Action Employer.*

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Industry Recruiter

Mr. Jensen has over 20 years of experience in human resource consulting and staffing for the biotechnology and pharmaceuticals industry.

Adviser Bill Lindstaedt
Director,
UCSF Career Center

Mr. Lindstaedt has been providing career related advice to scientists and engineers for nearly 15 years, with a particular emphasis on working with graduate-level trainees in the life sciences.

Adviser Naledi Saul
Assistant Director,
UCSF Career Center

Ms. Saul has 7 years of career counseling with 4 years focused on counseling graduate students and postdocs in the biomedical and health sciences. Her forte is working with scientists pursuing careers in the public health arena.

Adviser Jim Austin
Editor, Science's
Next Wave

Dr. Austin has a Ph.D. in physics and worked in academia before coming on board to write about traditional and nontraditional career paths for scientists.

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POSITIONS OPEN

POSTDOCTORAL FELLOWSHIPS
Cardiopulmonary Genomics Program
University of Maryland
School of Medicine

The newly formed Cardiopulmonary Genomics Program is seeking Postdoctoral Fellows for research in the genomics, molecular biology, signaling, and pharmacology of G-protein coupled receptors. The ideal candidate (Ph.D., M.D., D.V.M., or equivalent) will have published experience in DNA manipulation and related techniques, human polymorphism discovery, receptor signaling, promoter analysis, and production of transgenic mice. An interest in heart and lung disease is helpful but not required. For examples of research and publications perform National Library of Medicine search on Liggett SB. The program is located in new, well-equipped, state-of-the-art laboratory space at the medical campus in Baltimore, Maryland.

Individuals interested should send their curriculum vitae to: **Stephen B. Liggett, M.D., University of Maryland-Baltimore,** via **e-mail: sbliggett@gmail.com.**

The University of Maryland-Baltimore encourages women and members of minority programs to apply and is an Affirmative Action/Equal Employment Opportunity/ADA Employer.

FACULTY CHEMIST, MOLECULAR IMAGING

Applications are invited for a position as an **ASSISTANT PROFESSOR** in the Department of Radiology and Radiological Sciences, Vanderbilt University, to establish and lead a research program in molecular imaging and targeted contrast agents within the Vanderbilt University Institute of Imaging Science. The successful applicant should have broad interests in the development of new agents for use with imaging by MRI, optical, nuclear, or other techniques, for applications in animal models and/or with translational potential for clinical medicine. The program in molecular imaging will be housed in new laboratories under construction and will support the activities of a large group of imaging scientists and collaborators working with different modalities as well as with nanotechnology. Applicants with relevant experience and strong qualifications in chemistry, biochemistry, or similar should send their curriculum vitae and arrange for two references to be sent to: **Dr. John C. Gore** at **e-mail: john.gore@vanderbilt.edu.** *Vanderbilt University is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL FELLOW

A Postdoctoral Fellow position is immediately available to study the molecular mechanisms associated with mood disorders. The candidate should be a Ph.D. with demonstrated experience in reverse transcription polymerase chain reaction, microarray, short interfering RNA, and other molecular biological techniques. Preference will be given to those who have prior experience with human subjects and/or rodents. Send your curriculum vitae along with three references to: **Yogesh Dwivedi, Ph.D., Department of Psychiatry, University of Illinois at Chicago, 1601 West Taylor Street, Chicago, IL 60612. E-mail: ydwivedi@psych.uic.edu.** *University of Illinois at Chicago is an Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL FELLOW POSITION, Northwestern University, Chicago, requiring two years of experience in advanced molecular techniques and cell/tissue imaging, to study podocytes in diabetic kidney disease (see: *Diabetes* **53:**2939–49, 2004). Animal experience preferred, for ongoing in vivo studies. Guaranteed funding for two years. Send curriculum vitae to **e-mail: sheldon-chen@northwestern.edu.**

POSTDOCTORAL POSITION: NEURAL STEM CELLS

Two Postdoctoral positions are immediately available to work on normal differentiation and cancer of neural stem cells involving cell biology, transgenic/knockout mouse models, and chromatin structure. For examples of recent research projects, see: *Genes Dev.* **18:**889–900, 2004; *Mol. Cell. Biol.* **24:**8018–8025, 2004; *Mol. Cell. Biol.* **21:**5531–5540, 2001; *Nature Med.* **6:**826–831, 2000; *Mol. Can. Ther.* **4:**343–349, 2005; or at **website: http://www3.mdanderson.org/~genedev/majumder.html.** Please send curriculum vitae and names and addresses of three references to: **Sadhan Majumder, Department of Molecular Genetics, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 1006, Houston, TX 77030. Telephone: 713-834-6347; e-mail: majumder@mdanderson.org.**

The University of Texas M.D. Anderson Cancer Center is an Equal Opportunity Employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability, or veteran status except where such distinction is required by law. All positions at The University of Texas M. D. Anderson Cancer Center are security sensitive and subject to examination of criminal history record information. Smoke-free and drug-free environment.

The Institute of Environmental Health Sciences at Wayne State University is recruiting for a Director of the Community Outreach and Education Program at the M.S. (**RESEARCH ASSOCIATE**) or Ph.D. (**ASSISTANT/ASSOCIATE PROFESSOR [RESEARCH]**) level. Qualifications include knowledge/experience with community outreach involving diverse community groups, preferably with a focus on environmental health and science education. Excellent written/verbal communication, organizational/managerial, and computer skills are essential. Position and salary are commensurate with degree and experience. Send application and the names of three references to:

Search Committee
c/o Ms. Karen Carty
Wayne State University
Institute of Environmental Health Sciences
2727 Second Avenue
Room 40000
Detroit, MI 48201
Telephone: 313-577-0100
E-mail: k.carty@wayne.edu

Wayne State University is an Equal Opportunity Employer.

POSTDOCTORAL POSITION

A full-time, Postdoctoral position is available in the Department of Pathology, Medical College of Wisconsin. The successful candidate will participate in a study of human malignant tumor markers, to verify and characterize a group of candidate molecules identified from human malignant tumor tissues. Qualified applicants should have a Ph.D. or equivalent degree in molecular biology/cell biology/biochemistry or related biomedical science disciplines. The position is available immediately. Please submit curriculum vitae to: **Rongshan Li, M.D., Ph.D., Department of Pathology, Medical College of Wisconsin, Milwaukee, WI 53226. E-mail: rli@mcw.edu.**

Medical College of Wisconsin is an Equal Opportunity Employer.

POSTDOCTORAL RESEARCH FELLOWSHIP, University of California, San Diego Laboratory for Neurogenetics, Department of Neurosciences. Utilize mouse and human genetics, cell biology, and advanced imaging techniques to study neurological development in relationship to disease. Ph.D. or M.D. in the biological sciences, with demonstrated expertise in molecular, cellular, and biochemical techniques. Competitive funding is available. See **website: http://gleesonlab.ucsd.edu.** Contact **e-mail: gleesonadmin@ucsd.edu.**

POSTDOCTORAL, RESEARCH, AND CLINICAL FELLOWSHIPS
at the
National Institutes of Health
U.S. Department of Health and Human Services

Website: http://www.training.nih.gov

NIH is dedicated to building a diverse community in its training and employment programs.

POSTDOCTORAL/RESEARCH ASSOCIATE POLYMER CHEMISTRY

Research will focus on the use of the sulfated polysaccharide carrageenan to develop novel vaginal formulations (microbicides) for prevention of AIDS and other sexually transmitted diseases. In addition to using chemical synthesis to develop novel formulations, the individual will be in charge of assaying existing formulations for stability, homogeneity, and quantitating the amounts of additional active ingredient(s) and excipient(s) in the formulation. The individual will need to be able to work independently, as well as with an enthusiastic group of virologists and technicians who are conducting biomedical aspects of microbicide product development. The individual will also have an opportunity to interact with suppliers and manufacturers of the Population Council's microbicide products. We are located on the campus of the Rockefeller University, which is part of the Tri-Institutions that includes Memorial Sloan Kettering and New York Weill Cornell Medical Center. Send letter of interest and curriculum vitae or address inquiries to: **Dr. David M. Phillips, Population Council, 1230 York Avenue, New York, NY 10021. Telephone: 212-327-8744; e-mail: dphillips@popcouncil.org.** *Equal Opportunity/Affirmative Action Employer.*

RESEARCH FELLOW, POSTDOCTORAL

A.I. duPont Hospital Laboratory of Human Genetics seeks a Postdoctoral Fellow. Must have Ph.D. in cell biology, biochemistry, genetics, or related and knowledge of immunology assays (Western Blot and immuno fluorescence); recombinant DNA methods (sequencing, mutagenesis, cloning, polymerase chain reaction, and reverse transcription polymerase chain reaction); protein expression and purification of recombinant proteins and from mammalian cells; cell biology techniques (transfection and generation of cell lines); and microscopy (light and fluorescence). Resume to: **A. M. Riddle, Adminstrator for Nemours Biomedical Research, A.I. DuPont Hospital for Children, P.O. Box 269, Wilmington, DE 19899.**

GRADUATE PROGRAM

PH.D. PROGRAM, MICROBIOLOGY AND CELL SCIENCE
University of Florida

The University of Florida offers an excellent doctoral program in Microbiology and Cell Science intended to develop young scientists who are prepared to take places in the scientific community as recognized leaders in their areas of expertise. Research areas include microbial physiology and biochemistry, metabolic engineering, biotechnology, environmental microbiology, molecular genetics, comparative genomics, bioinformatics, host-pathogen relationships, and immunology. Competitive stipends, fellowships, and assistantships are available to qualified applicants. To apply or obtain further information, visit **website: http://microcell.ifas.ufl.edu/.** *Women and minorities are encouraged to apply. The University of Florida is an Equal Opportunity Employer.*

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